Volume 16 Number 2

JOURNAL
of
DENTAL RESEARCH

FOUNDED BY WILLIAM J. GIES IN 1919

Official Publication of the

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

PROPERTY OF DENTAL LIBRARY UNIVERSITY OF MICHIGAN DON'T MUTILATE OR REMOVE

April, 1937

Anatomical and histological examination of the structure and development of the elephant molar. *Fritz Driak*, M.D. (University of Vienna, Austria) 73

Congenital bilateral hemangioma of the cervico-facial region. Preliminary report. *Frederic W. Bancroft*, M.D., F.A.C.S., *C. Zent Garber*, M.D., and *Malcolm W. Carr*, D.D.S., F.A.C.D. (Fifth Avenue Hospital, New York City) 81

The chemical nature of the inorganic portion of fetal tooth substance. *Marian L. LeFevre*, M.S., *William F. Bale*, Ph.D., and *Harold C. Hodge*, Ph.D. (University of Rochester) 85

Growth of the palate in the human fetus. *Benjamin Freiband*, M.S. (New York University and American University of Beirut, Syria) 103

Gold decorated teeth from the Philippine Islands. *Leslie F. Rittershofer*, D.D.S., M.S. (Ann Arbor) 123

Round-cell sarcoma of the mandible. *Paul C. Kitchin*, M.S., D.D.S., and *Charles A. Doan*, M.D. (Ohio State University). 135

The Cape-Kitchin modification of the celloidin decalcifying method for dental enamel. *Charles F. Bödecker*, D.D.S., F.A.C.D. (Columbia University) 143

Salivary cholesterol. *Frances Krasnow*, A.M., Ph.D., and *Edith Oblatt*, A.B. (Guggenheim Dental Clinic) 151

Microbiology of gingival inflammations. *Theo. B. Beust*, D.D.S., M.D., F.A.C.D. (University of Louisville) 157

PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORIAL OFFICE: 260 Crittenden Blvd., Rochester, N. Y.

Copyright, 1937, by The International Association for Dental Research

Made in United States of America

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee: J. Frank Hall, Maynard K. Hine, Harold C. Hodge, M. W. McCrea and Hamilton B. G. Robinson.

Managing Editor—Hamilton B. G. Robinson. *Advertising Manager*—J. Frank Hall *Circulation Manager*—M. W. McCrea. *Treasurer*—Harold C. Hodge. *Secretary*—Maynard K. Hine.

Additional members of the Board of Editors—see sectional representatives named in the fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1936–'37)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
Austria	Vienna	Fritz Driak	Balint Orban	Bernhard Gottlieb
Canada	Halifax	Hugh M. Eaton	J. S. Bagnall	S. G. Ritchie
	Toronto	A. W. Ellis	A. J. McDonagh	A. J. McDonagh
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
China	Chengtu	R. Gordon Agnew	R. Gordon Agnew	R. Gordon Agnew
Czechoslovakia	Prague	Friedr. Neumann	Friedr. Neumann	Jan Jesensky
England	London	Evelyn Sprawson	Evelyn Sprawson	Evelyn Sprawson
Hungary	Budapest	Ferenc Köszeg	Josef Szabo	Josef Szabo
South Africa	Johannesburg	W. A. Wilson	Geoffrey Friel	J. C. M. Shaw
United States	Ann Arbor	U. G. Rickert	U. G. Rickert	U. G. Rickert
	Baltimore	M. S. Aisenberg	M. S. Aisenberg	A. H. Schultz
	Boston	F. W. Morse	L. M. S. Miner	P. E. Boyle
	Chicago	E. D. Coolidge	H. J. Noyes	Emil Mueller
	Cleveland	S. W. Chase	Thomas J. Hill	S. W. Chase
	Columbus	Paul C. Kitchin	Paul C. Kitchin	C. O. Boucher
	Iowa City	Charles L. Drain	A. W. Bryan	George S. Easton
	Louisville	Raymond E. Myers	T. B. Beust	T. B. Beust
	Minnesota	Harold G. Worman	Carl J. Grove	W D. Armstrong
	New Haven	James P. Pigott	A. W. Crosby	S. S. Arnim
	New York	Theodor Rosebury	L. M. Waugh	Theodor Rosebury
	Philadelphia	Thomas J. Cook	Thomas J. Cook	J. L. T. Appleton
	Pittsburgh	L. E. Van Kirk	H. E. Friesell	L. E. Van Kirk
	Richmond	G. Van Huysen	Harry Bear	G. Van Huysen
	Rochester	M. W. McCrea	R. E. Brawley	B. G. Bibby
	San Francisco	Ingo Hackh	J. A. Marshall	J. A. Marshall
	St. Louis	C. O. Simpson	Geo. B. Winter	R. C. Wheeler
	Washington	G. C. Paffenbarger	Wilmer Souder	H. E. Harvey

SOCIETIES THAT ACTIVELY SUPPORT THE JOURNAL OF DENTAL RESEARCH, AND THEIR REPRESENTATIVES IN THE HONORARY BOARD OF EDITORS

American Dental Association.....LEROY M. S. MINER
New York Academy of Dentistry.....ARTHUR H. MERRITT
American College of Dentists.....ALBERT L. MIDGLEY, Providence
First District Dental Society of the State of New York.....JOHN OPPIE MCCALL
Academy of Stomatology (Philadelphia).....JOHN BURKHARDT, JR.
Allied Dental Council (New York).....LEON HARRIS
American Academy of Dental Science.....CHARLES E. HATCH, Boston
American Stomatological Association:
H. J. LEONARD AND EDWARD FRANKEL, JR., New York City
Kings County (N. Y.) Dental Society.....LEONARD KOHN
Odontographic Society (Chicago).....HOUSTON F. METHVEN

Subscription price $5.00 per volume. Single numbers when available $1.00.

Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 1879

WAVERLY PRESS, INC.
BALTIMORE, U. S. A.

ANATOMICAL AND HISTOLOGICAL EXAMINATION OF THE STRUCTURE AND DEVELOPMENT OF THE ELEPHANT MOLAR[1]

FRITZ DRIAK, M.D.

Histological Department (Director, Prof. B. Gottlieb), Dental Institute (Director, Prof. H. Pichler), University of Vienna, Vienna, Austria

This report presents the results of a study of the molars of about twenty elephants of different ages, including both Indian and African elephants. Indian elephants are larger than African, and their molars are different in anatomical structure. African elephants are divided into the South and East African groups, with three toes on their hind legs, and the West African, with four toes. Both African types are represented in this study. Elephants have two tusks in the upper jaw, no incisors, and one molar on each side of the jaw in both lower and upper. *Fig. 1* shows the enamel folds of the molar of an African elephant. These folds resemble rhomboids, compressed in a mesiodistal direction. The chewing surface of the molars of the Indian elephant is more elliptical in shape, as seen in *Fig. 2*. The molars are composed of enamel plates or lamellae enclosing dentin, and connected with each other by cementum (*Figs. 1, 2*). It was formerly thought (Corse, 1799) that the single lamellae are to be considered as individual teeth only secondarily connected with one another by cementum to form a complete tooth; but each of these single lamellae is itself composed of cone-shaped tips, which are in turn connected by cementum.

According to Corse the first molar has 4 to 8 lamellae, and the succeeding molars more and more. He thought that the Indian elephant had 7 to 8 molars during its lifetime. Some authors speak of deciduous molars, premolars, and permanent molars in the ele-

[1] Read at the monthly meeting of the Society of Austrian Dentists, Vienna, February 7, 1934. See also proceedings of the International Association for Dental Research: *J. Den. Res., 13:* 213, 1933.

phant, and many attempts have been made to distinguish deciduous and permanent dentitions in this animal. Corse, and Röse pointed out that there is no more biological justification for such distinction in the teeth of elephants than there is in the teeth of fishes or reptiles which have large sets of tooth germs ready to develop if the corresponding functioning tooth is shed. Likewise in sharks successive series of teeth develop in the jaw and come into place if the functioning teeth fall out. In such cases development and eruption of the teeth occur in an occlusal direction; in the elephant development and eruption proceed horizontally from distal to mesial. In the first teeth of elephants, when the animal is small, the teeth have few lamellae. As the animal grows larger the succeeding teeth increase

Fig. 1

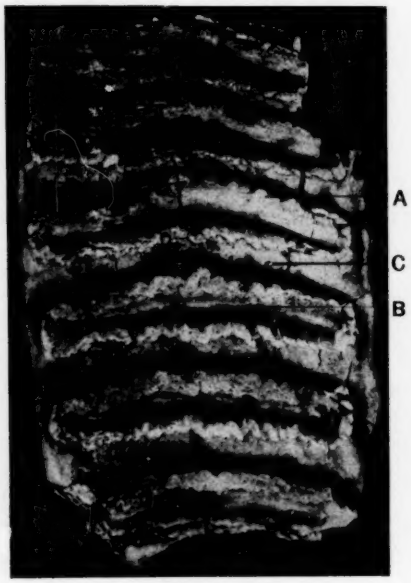

Fig. 2

Fig. 1. Occlusal surface of African elephant molar. Enamel folds (a) are rhomboid in shape, compressed mesio-distally; they enclose dentin (b), and are connected with one another by cementum (c). Original length of occlusal surface—21 cm., width—6 cm., weight of specimen—6 lb.

Fig. 2. Occlusal surface of Indian elephant molar. (a) enamel folds; (b) dentin; (c) cementum.

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 3. Roentgenogram of lower jaw of newborn Indian elephant. (a) first molar; (b) second molar with 8 lamellae; (c) third molar with lamellae disarranged, calcified union having not yet occurred.

Fig. 4. Mesial part of second molar shown in *Fig. 3.*

Fig. 5. Roentgenogram of lower jaw of 11-months Indian elephant embryo. First and second molars are shown; third molar does not appear, being as yet uncalcified. The lamellae, maintained by soft tissue, are shown in proper relationship. Length of original specimen—12 cm., length of embryo—56.5 cm.

Fig. 6. Roentgenogram of lower jaw of Indian elephant 6 months old. (a) first molar with 2 roots; (b) second molar also showing 2 roots and with interconnected lamellae, erupted and worn down mesio-occlusally.

Fig. 7. Roentgenogram of lower jaw of middle-aged Indian elephant, showing fourth molar in contact with distal end of third (a). Mesial part of fourth molar (b) is erupted and in function, its distal part (c) not yet fully developed. This tooth also shows 2 roots: a thin mesial (d) and wide distal (e), divided by a bony septum (f).

both in size and in number of lamellae. Only one molar or two half-molars are in function at a given time. As the medial part of a tooth is shed the next molar begins to erupt; in this way a functioning tooth is present continuously throughout life. It is now accepted, therefore, that there is only one dentition in the elephant; individual molars follow one another in succession, and the number of lamellae increases as the animal grows older. De Terra described the first molar of the Indian elephant as composed of 4 lamellae, the second of 8, the third of 12, the fourth of 12, the fifth of 16, and the sixth of 24 to 27. The lamellae of African elephants are less numerous but wider.

In the present study the development of elephant molars was observed with the aid of roentgenograms of 18 jaws. *Fig. 3* shows the lower jaw of a newborn Indian elephant. The first molar (a) has two roots and a crown with three enamel tips. Behind this small tooth are seven lamellae, the first four fused together below (*Fig. 4*), and the last three entirely separate. The lamellae, consisting of dentin covered first with enamel and then with cementum, are built up as finger-like or cone-shaped processes, described in the literature as *digitelli* or *denticuli*. The first and second molars are separated by a bony septum, and another bony septum separates the second molar from the third. The lamellae of the third molar appear disarranged; but they are not yet connected with one another by calcified tissue and have therefore lost their support during maceration and drying of the specimen. A roentgenogram of the lower jaw of an 11-month embryo Indian elephant is shown in *Fig. 5*.[2] Here the lamellae stand in perfect order because they are connected by the soft tissues of the tooth germ, which in this instance have not been lost by maceration. This specimen shows that all the lamellae of the same tooth develop from a single tooth germ, and are thus not individual teeth but parts of the same tooth.

The appearance of two definite roots in the small first molar shown in *Fig. 3*, suggested a study of the formation of roots in the other molars, which at first seemed to be rootless. But *Fig. 6*, a roentgenogram of the lower jaw of an Indian elephant 6 months old, clearly

[2] This specimen has been sectioned and studied microscopically, and will be described in a separate report.

shows the progress of root development. In *Fig. 6* the first molar is more fully erupted. One part of the crown is worn down by mastication. The two roots are well developed. The mesial part of the second molar is also erupted, and the mesial 4 lamellae are also worn down by mastication. All 8 lamellae are joined in a single tooth with two roots similar to those of the first molar partly formed, the mesial thinner than the distal, and the two divided by a bony septum. Similar root formation has been observed in all the succeeding molars. *Fig. 7* shows the lower jaw of a middle-aged Indian elephant. The molar, which may be the fourth, has about 16 lamellae; and root formation is plainly seen. The smaller mesial root is divided by a septum from the wider distal root. In *Fig. 8*, the lower jaw of a very old Indian elephant, a molar with about 24 lamellae also presents two roots, a thinner mesial and a very wide distal root. African elephants show similar root formation in the molars; but in these, although a thin mesial root is present, instead of one wide distal root there are several smaller roots. The regular formation of roots in elephant molars has not been previously described.

Several authors have observed that elephants shed their molars once in about 10 to 15 years. Shedding occurs six times during the life of an elephant. Six molars develop in one side of a jaw, so that the elephant has 24 molars during its life. It is very difficult to make exact observations of this kind, because elephants live about 150 years, and at least two human lifetimes would be required to observe the shedding of the teeth of one elephant. Any other means of observation involves the possibility of error. Tooth germs seem to be present in the jaw continuously, and the number of successive teeth depends only on the length of life of the animal. The biologic constitution of the elephant denture is such that the animal can never become edentulous. Its two different types of teeth are always in function: the tusk is persistently growing, newly formed continuously at its apical end; while the molars are shed mesially as they are formed distally. Magitot and Röse thought that the single lamellae of one molar are separate teeth, which become united only secondarily by cementum—the so-called concrescence theory. Miller and Dieck thought (1900) that the enamel folds of one molar are connected primarily; but they did not fully amplify their view. Bolk

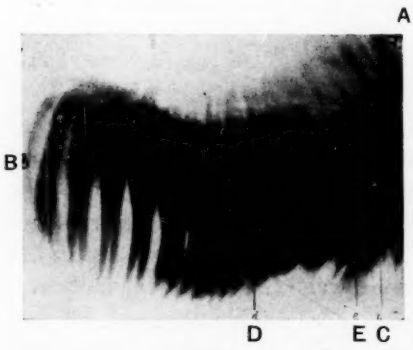

Fig. 8

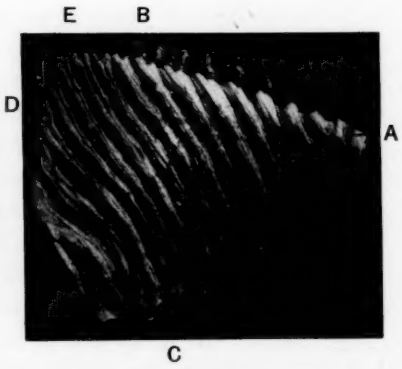

Fig. 9

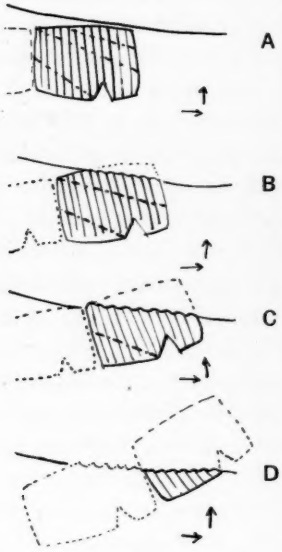

Fig. 10

Fig. 11

Fig. 8. Roentgenogram of lower jaw of a very old Indian elephant. (a) mesial end of tooth in function; (b) distal end of unerupted tooth; (c) small mesial root; (d) wide distal root; (e) interradicular bony septum.

Fig. 9. Longitudinal section of molar of an old Indian elephant. Triangular shape due to occlusal wear at mesial end (a). (b) occlusal surface; (c) root region; (d) distal end with unerupted lamellae covered with cementum; (e) distal end of occlusal surface where lamellae are longest, being as yet unworn.

Fig. 10. Diagram showing eruption of the elephant molar. Rectangular before eruption (a), a triangular area is worn away as mesial edge erupts (b); process of eruption and mesial shifting continues as tooth becomes triangular (c, compare *Fig. 9*), and is eventually worn away as next tooth comes into function (d).

Fig. 11. Elephant molar dentin, high magnification, showing waved course and numerous branches of tubuli.

(1919) was the first to investigate an elephant embryo of 20 cm. length. He concluded that a single molar is built by one and the same enamel organ, and proved the concrescence theory wrong. The 11-month elephant embryo reported in the present paper is 56.5 cm. long, and therefore shows more than Bolk's specimen; the rela-

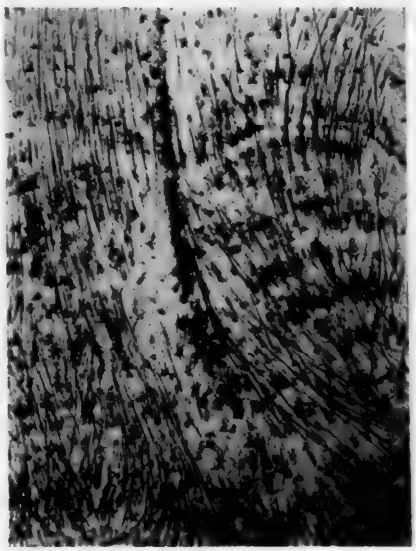

Fig. 12 *Fig. 13*

Fig. 12. Dentino-enamel junction of elephant molar. (a) dentin; (b) enamel, showing scale-shaped rods and Schreger's lines.

Fig. 13. Elephant molar cementum, with numerous cementum corpuscles and connective tissue fibers.

tion of the enamel organ to the tooth lamellae in it is the same as that described by Bolk.

To investigate the process of tooth eruption in the elephant, a molar of an old Indian elephant was cut into sagittal halves, and the sectioned surface of one half polished; this specimen is shown in *Fig. 9*. The length of the individual lamellae is about the same throughout the molar before eruption (*Fig. 8*); the differences in lamellar length shown in *Fig. 9* are the result of wear. An elephant molar is shown diagrammatically in *Fig. 10a*. The tooth moves

mesially and occlusally until its mesio-occlusal edge erupts and is worn down by mastication (*Fig. 10b*). The process of eruption continues; the tooth substance is worn down on the occlusal surface, while the mesio-occlusal edge moves mesially and occlusally; and the tooth becomes triangular (*Figs. 10c, 9*). During this process the mesial edge of the tooth is progressively lost (*Fig. 9a*), and the occlusal surface progressively worn, so that the form shown in *Fig. 10d* is eventually produced; at the same time the succeeding tooth comes into the occlusal relation formerly occupied by the previous tooth, as shown in *Fig. 10b*. As eruption progresses the little triangular piece of tooth becomes entirely cast off and the next tooth takes its place.

Fig. 9 shows the triangular elephant molar. On its mesial edge (a) the lamellae are short, while at (e) they have their full length. The former must once have had the same length. The occlusal surface (b) shows the enamel folds elevated, the cementum and dentin depressed by greater relative wear. The lamellae (d), still covered with cementum, have not yet erupted. On the mesial area of the root part of the molar resorption is taking place, facilitating shedding of the tooth. From the other half of the tooth shown in *Fig. 9* ground sections of smaller areas were prepared for microscopic examination. *Fig. 11* is a high magnification of the dentin showing typical mammalian characteristics. The dentinal tubuli show slightly waved courses and numerous branches which often anastomose. The enamel (*Fig. 12*) consists of enamel rods of the same scale-like shape as in other animals and in man. Groups of longitudinal rods alternate with groups of transverse rods, forming the so-called Shreger's lines. The dentino-enamel junction is a straight line, across which some dentinal tubuli enter the enamel. The cementum (*Fig. 13*) shows very numerous cementum-corpuscles, and near its surface connective tissue fibres which appear to be the same as Sharpey's fibres. These fibres are missing in cementum connecting single tooth lamellae with one another.

CONGENITAL BILATERAL HEMANGIOMA OF THE CERVICO-FACIAL AREA[1]

Preliminary Report

FREDERIC W. BANCROFT, M.D., F.A.C.S., C. ZENT GARBER, M.D., and MALCOLM W. CARR, D.D.S., F.A.C.D.

Department of Surgery, Fifth Avenue Hospital, New York City

A white female, seven weeks of age, presented with swelling of both cheeks, present since birth, and ulceration of the lower lip discovered two weeks after birth. The family history and past medical history were entirely negative. Examination revealed prominent swellings in both cheeks, the left larger than the right. The swelling on the left side extended from the zygomatic arch and malar bone to the lower border of the mandible, with some diffuse extension into the tissues of the neck. Postero-anteriorly, it extended from just anterior to the ear and the angle of the mandible to the commissure of the mouth. In the postero-anterior dimension the swelling appeared separated into two portions; the posterior overlying the masseteric fascia, the more distended anterior occupying the cheek region, from the masseter to the commissure of the mouth and the ala of the nose, limited above by the inferior border of the orbit. The appearance of the right side of the face corresponded in a general way to the left. Telangiectasis was pronounced in both cheeks and extended into the tissues of the neck. There was a small encrusted ulcer 0.5 cm. in diameter in the center of the anterior portion of the swelling of the left cheek. The general appearance of the swelling was that of extreme distension with dilatation and engorgement of superficial terminal vessels; the skin being tense and red. There were several areas, 1 cm. in diameter, that appeared purplish or bluish in color. Upon palpation the mass was soft and compressible and gave a distinct sense of

[1] Presented at the Thirteenth General Meeting of the International Association for Dental Research, Chicago, March 16–17, 1935: *J Den. Res., 15:* 206, 1935.

fluctuation over a considerable area. Pressure applied to the swelling did not elicit pitting and caused no subjective symptoms. The swelling extended through the entire thickness of the cheek bulging the buccal mucous membrane within the mouth and seeming to prevent complete closing of the mouth. A large, deeply excavated, sloughing ulcer on the lower lip was 2 cm. in diameter. The center of the ulcer was situated to the left of the median line and occupied the greater portion of the left side of the lip, extending through the vermilion border but not to the inner surface of the lip. Intra-oral examination revealed several small superficial ulcers (aphthae) situated upon the alveolar and buccal mucosa. There was no ulcer in the buccal mucosa on the left side corresponding to the position of the external ulcer. The floor of the mouth was elevated although not inflamed and the tongue was pushed slightly back. Salivary secretion was normal or slightly hyperactive. Throat examination was negative. The submaxillary and submental lymph nodes were palpable on both sides.

Both ear canals were narrowed by external pressure on the floor and posteriorly although the obstruction was soft so that an applicator could be passed easily. The drums could not be seen. There was a small amount of purulent discharge from both ears. Mastoid and nasal examinations were negative. Eye examination elicited normal reaction to light and accommodation with no strabismus, nystagmus, ptosis or exophthalmos. Physical examination was otherwise essentially negative for any abnormalities of function, growth or development.

Roentgenograms taken of the skull and of the bones of the face in the lateral direction show no evidence of bone disease. Hematology and urinalysis were essentially normal. Smear from ulceration on lip was negative for fusospirochetes and a throat culture was negative for bacillus diphtheria. Vaginal smear showed many gram positive and gram negative cocci, occasional pus cells, and no gram negative intracellular diplococci. Wassermann reaction was negative.

The patient was under observation for eight weeks. Treatment was directed essentially at the general care of the infant, with special consideration being given to diet and nutrition. It was generally conceded that attempt at any form of local treatment would be of little avail. Biopsy was not done. For a period of five weeks the

condition extended gradually, continually invading the deeper tissues of the neck (*Figs. 1 and 2*). The sixth week of observation was notable because of a period of remission of acute symptoms accompanied by remarkable general improvement, not attributable to any known cause. This one-week period of remission was followed, however, by a period of recrudescence during which there was an acute exacerbation of all symptoms, terminating fatally at the end of the eighth week of observation. The tumor mass had infiltrated tissue over an extensive area and developed to enormous proportions as shown at the postmortem examination. Microscopically the tumor was diagnosed as hemangioma.

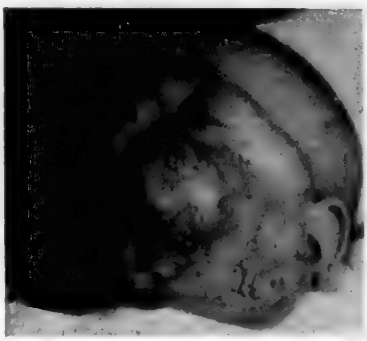

Fig. 1

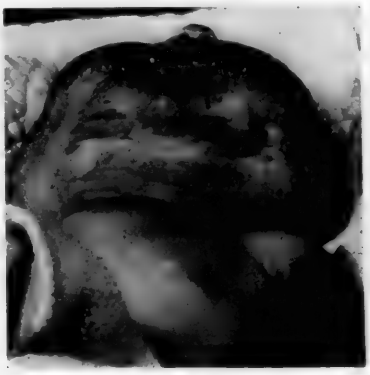

Fig. 2

Fig. 1. Photograph of left side of face taken three weeks before death. Note well defined limitations of extreme distension and extensive telangiectasis. Right side presented similar appearance. Ulcers on lower lip and left cheek have healed while vermillion portion of lower lip has been destroyed by ulceration. Cicatricial contraction at angles of mouth, together with bilateral pressure caused by tumor mass and distension of floor of mouth, cause lips to appear puckered and prevent child from bringing lips together in normal position.

Fig. 2. Photograph taken three weeks before death, showing bilateral extension into neck. Note enormous overgrowth and distension of tissue. Width of tumor mass actually exceeded width of thorax.

THE CHEMICAL NATURE OF THE INORGANIC PORTION OF FETAL TOOTH SUBSTANCE[1]

MARIAN L. LEFEVRE, M.S., WILLIAM F. BALE, PH.D., AND HAROLD C. HODGE, PH.D.[2]

From the Departments of Biochemistry and Pharmacology, and Medicine (Radiology), School of Medicine and Dentistry, University of Rochester, Rochester, New York

Analyses of fetal teeth were made in 1862 by Hoppe-Seyler (1) who determined the mineral content of enamel from the teeth of three term fetuses and found the calcium content to average 31.4%, phosphorus 13.9%, with a Ca:P per cent ratio averaging 2.26. In 1931, Hess, Lewis and Roman (2) concluded from a radiographic study of tooth calcification from birth to adolescence that calcification of permanent teeth is a post-natal phenomenon and that, taken altogether, deciduous teeth prior to birth are calcified in amounts corresponding to less than one-half gram of tricalcium phosphate. These authors found, by pooling corresponding teeth from opposite sides of three infant jaws, that calcium ranged in amounts from 3.78 to 17.18 mgm. and phosphorus from 1.82 to 8.67 mgm. for single teeth, with an average Ca:P per cent ratio of 2.04.

In view of the small number of analyses made on fetal teeth we have attempted to determine the nature of the tooth substance within the fetal jaw, and to compare it with other calcified structures both in the fetal and in the mature organism.

METHODS

The procedure for chemical analysis of fetal teeth was adapted from methods used by Crowell, Hodge, and Line (3) for permanent teeth. The teeth, after removal from the fetal jaws at autopsy, were immersed in tap water until chemical determinations were made. In order to obtain samples large enough for analysis, the teeth were pooled in pairs, each pair consisting of the corresponding teeth from opposite sides of the same jaw. The method of extraction of organic matter was that adapted from Gabriel (4) by Crowell et al. The inorganic residue

[1] Presented at the Thirteenth General Meeting of the International Association for Dental Research, Chicago, March 16–17, 1935; J. Den. Res. 15: 165, 1935.

[2] This work was supported by a grant from the Rockefeller Foundation.

of the tooth was dissolved in a known amount of standard 0.2 N HCl, the volume made to 100 cc., and the "residue solution number" determined by titrating aliquots with standard NaOH(4). Standard micro methods have been used for the determination of Ca(4), Mg(4), P(4) and CO_3(5).

DATA

The data obtained in the chemical examination of fetal teeth are given in the tables.[3] A, B, C, D, and F were term fetuses; E was a premature seven-month fetus. A conventional notation was used for the teeth (6), i.e. $\underline{a}|\underline{a}$ represents the pooled right and left upper central incisors. In no case was the first permanent molar sufficiently calcified to yield material for chemical investigation.

In Table I, part 1, and Table II are given the values found in the chemical determinations; in Table I, part 2, the results of a statistical study of the data (excepting the data in Table III). Values were discarded where the deviation from the average was greater than 2.5 times the standard deviation. The numbers of atypical values discarded on this basis were: moisture, 2; inorganic residue, 2; Ca, 1; Mg, 0; P, 1; residue solution number, 3; CO_3, 0; and Ca:P per cent ratio, 2.

The per cent values for Mg, moisture, and residue solution number vary between wide limits, viz., Mg from 0.24 to 2.56—average 1.12; moisture from 3.85 to 10.4—average, 6.82; residue solution number from 8.9 to 17.8—average 13.4. The limits for per cent inorganic residue are narrower, from 72.3 to 91.6—average 80.4. The per cent CO_3 values are relatively constant, from 3.16 to 3.46—average 3.30. The values for Ca and P, and hence the Ca:P per cent ratio, show the least variability, Ca ranging from 29.0 to 36.7—average 34.5; P, 16.0 to 18.1—average 16.9; and the ratio, 1.76 to 2.27—average 2.04.

In brief, the data may be represented as the average per cent plus or minus the standard deviation as follows: moisture—6.82 ± 2.0; inorganic residue—80.4 ± 4.5; Ca—34.5 ± 2.3; Mg—1.12 ± 0.6; P—16.9 ± 0.5; residue solution number—13.4 ± 2.4; CO_3—3.3 ± 0.1; and Ca:P per cent ratio—2.04 ± 0.2.

In the term fetus, the moist weights of the calcified portions of the various teeth, i.e. incisors, canines, molars, vary in an irregular fashion.

[3] The authors wish to express their appreciation to Grant Van Huysen for securing the fetal teeth at autopsy.

The upper central incisor is the heaviest tooth, the upper first molar next, and the lower first molar next. From fetus to fetus, the same relative weights are found for the various teeth, thus giving a jagged graph of weights against tooth position. The lower jaw pattern is essentially the same as that for the upper jaw, but each tooth is consistently lighter than the corresponding tooth in the upper jaw (*Fig. 1*—averages from data in Tables I and III).

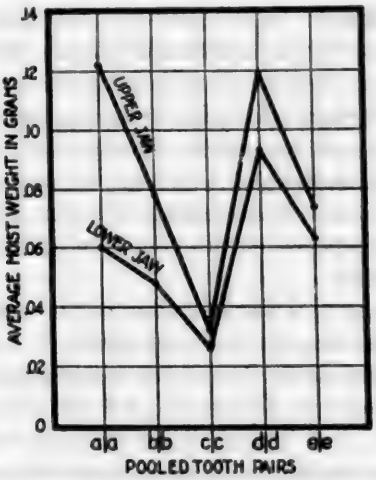

Fig. 1. Average moist weights (g.) of pooled pairs of teeth from five-term fetuses are plotted along ordinates against pooled tooth pairs, a | a being the central incisors, etc. Note similarity in pattern for upper and lower jaws. Patterns for each fetus follow relations shown by averages.

DISCUSSION

The discussion considers an interpretation of the data in two phases: (1), the nature of the inorganic tooth substance found in the fetal jaw (this includes normal values for constituents and individual variation); (2), comparison of this substance with tooth and bone substance of the mature organism, both as to per cent composition and molecular constitution.

The unstarred values in Table I, part 1, represent the normal values found for fetal tooth substance. When these values are plotted against individual teeth, in 80% of the cases there is a greater inor-

ganic weight per cent for central incisors both upper and lower than for the rest of the teeth. This condition is to be expected, since dental calcification is known to take place first here. It should be noted that the inorganic residue is expressed as per cent of dry weight of the sample. Thus a higher per cent inorganic residue means a higher degree of calcification; therefore, less organic as compared to inorganic substance in the solid part of the tooth. A higher per cent inorganic residue does not necessarily attend a larger weight of tooth sample (see *Fig. 1* and compare Table I, part 1). Concerning the rest of the data, there is apparently no correlation between the tooth type (morphologically) and its individual inorganic constituents. The moisture content varies between wide limits and is apparently not a function of age, nor does it show consistent variation from tooth to tooth nor from individual to individual. The magnesium content also varies widely but around different averages for different individuals. Values for calcium vary between narrow limits and phosphorus values are very nearly constant.

Progressive changes of the inorganic tooth substance within the fetal jaw are few, as shown by comparing data for a seven-month fetus with those for a term fetus. From the total inorganic weights of the tooth substance from term fetuses A and B of 0.4376 grams and 0.6260 grams, respectively, and from the seven-month fetus of 0.2387 grams, it is indicated that calcification increases between two and three times from the seventh to the ninth month of fetal life. The values of the individual inorganic constituents show no indication of a progressive change in the calcified tooth substance in the fetal jaw.

If fetal teeth are compared with values of other investigators for fully erupted deciduous and permanent teeth, several differences are notable; first, in moisture content; second, in variability of calcium values; third, in carbonate content; and fourth, in relative amounts of calcium present and, therefore, in Ca:P per cent ratio.

First, when compared with permanent teeth investigated by Crowell et al. (3) and others (see Table I, reference 2), fetal teeth show a lower moisture content. This is contrary to what might be expected, since the water content of fetal tissues is usually higher than that of adult tissues. Inasmuch as the infant tooth has a larger surface area than a fully developed tooth in comparison to its total weight, this

discrepancy may be due to an excess loss of moisture from the tooth in rinsing with alcohol and ether before determining the moist weight. In order to check this statement, the teeth from three additional term fetuses were rinsed in alcohol and ether and allowed to dry for a few minutes and weighed. A definite relationship was found between moist weight and moisture per cent (Table III) even among teeth from the same individual, the larger teeth apparently having a greater moisture per cent than the smaller ones. The larger fetal teeth as well as the fully erupted permanent teeth have a smaller surface area in comparison to their weight than the smaller teeth, and therefore a smaller drying surface, which results in an apparently larger moisture per cent. This may account for the smaller moisture per cents found in fetal teeth when compared with fully erupted teeth, and also for the variable but consistent increase in moisture per cent with moist weight among fetal teeth from the same individual.

Secondly, the calcium and phosphorus content of infant teeth have narrow limits of variation. In comparison, Crowell et al. report wide variation in calcium content of permanent teeth, but constancy of phosphorus values. The constancy of both calcium and phosphorus values in the infant teeth may be due to the more nearly uniform conditions under which the fetal tooth is calcified. Fully developed teeth, either deciduous or permanent, have developed, in their later stages, under wide variations of diet and other conditions. The conditions under which calcification takes place in the fetus are fairly constant. Booher and Hansmann (7) have shown constancy in both ash and calcium per cents of tibias of eight normal term infants. A history of the mothers in these cases shows a range in age from fourteen to forty years and wide variation in dietary conditions; and it is stated that in the case of the younger mothers, calcification of their own skeletons would not have reached its maximum. The constant calcium content of the tibias shows that calcification took place normally in the fetus regardless of the state of calcification of the maternal skeleton. Hence the more constant percentages of calcium and phosphorus in fetal teeth are to be expected.

Thirdly, values in adult teeth for per cent CO_2 reported by Gabriel (4) and those found in this laboratory (unpublished) are higher than for fetal teeth. An increase in carbonate content with age has been

TABLE I

Part 1[1]

FETUS	TOOTH	MOIST WEIGHT	MOISTURE[2] (MOIST WEIGHT)	INORG. (DRY WGT.)	Ca (INORG. WGT.)	Mg (INORG. WGT.)	P (INORG. WGT.)	RESIDUE SOLUTION NUMBERS	$\frac{Ca}{P}$
		gm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
A	a|a	0.0977	6.76	78.3	33.8	1.00	17.2	14.7	1.97
	b|b	0.0642	5.62	75.2	34.0		16.9	14.2	2.01
	c|c	0.0350	6.28	75.0	30.5		17.3	13.7	1.76
	d|d	0.0932	5.69	76.6	33.8	1.52	16.6	10.8	2.02
	e|e	0.0598	6.02	75.4	34.7		16.8	13.6	2.03
	a|a	0.0491	6.92	80.3	34.6		17.1	11.2	2.01
	b|b	0.0430	6.52	77.2	34.3		17.0	13.2	2.01
	c|c	0.0270	6.29	72.3	35.4		17.2	8.9	2.06
	d|d	0.0760	6.32	74.4	34.9		16.8	12.0	2.08
	e|e	0.0677	6.60	76.0	34.8		17.1	11.6	2.02
B	a|a	0.1294	7.96	86.5	33.6	1.00	16.7	13.9	2.01
	b|b	0.0775	7.74	83.8	33.8		16.7	12.6	2.02
	c|c	0.0551	9.98	78.9	34.3		16.4	21.0*	2.09
	d|d	0.1291	14.32*	79.3	34.2	1.24	16.6	14.1	2.06
	e|e	0.0871	9.29	80.4	34.3	1.53	16.9	10.8	2.03
	a|a	0.0640	6.25	89.0	33.7	2.56	17.3	11.5	1.93
	b|b	0.0561	8.20	83.8	34.3		17.2	13.5	1.99
	c|c	0.0449	9.81	80.4	33.8	1.56	16.9	12.3	2.00
	d|d	0.1149	4.53	80.8	35.1		17.2	12.7	2.04
	e|e	0.0866	8.89	80.3	34.9	0.932	17.2	11.5	2.03
C	a|a	0.1384		81.6	34.5	0.43	16.5	20.0*	2.09
	b|b	0.0776	7.73	78.3	35.2	0.75	16.8	13.5	2.09
	c|c	0.0371	6.19	79.9	29.0		16.3	11.4	1.78
	d|d	0.1444		80.1	35.1	0.24	16.3	13.5	2.15
	e|e	0.0976	7.68	80.8	35.8	0.62	16.7	15.6	2.14
	a|a	0.0681	7.07		35.1		16.9	17.2	2.08
	b|b	0.0540	7.04	79.1	35.2		16.6	17.8	2.12
	c|c	0.0294	5.78	79.9	20.4*		17.0	20.6*	1.20*
	d|d	0.1115		80.7	35.2		17.4	17.5	2.02
	e|e	0.0819	6.59	80.8	35.2		17.8	14.8	1.98
D	a|a	0.1678		83.5	35.8		18.1	15.3	1.98
	b|b	0.0973	12.70*	79.7	35.5		17.5	14.6	2.03
	c|c	0.0366	7.38	79.7	35.5			16.9	
	d|d	0.1415		79.8	35.6		17.0	14.5	2.09
	e|e	0.0793	6.56	80.7	36.7		16.0	15.9	2.27
	a|a	0.0828	8.21	85.7	35.0		16.2	15.2	2.16
	b|b	0.0654	9.02	80.5	36.0		16.6	13.8	2.17
	c|c	0.0294	5.78	81.0			16.7	11.5	
	d|d	0.1042		80.2	35.9		16.6	12.8	2.16
	e|e	0.0607	5.77	79.6	35.7		14.2*	12.5	2.52*

TABLE I—*Concluded*

Part 1[1]—*Concluded*

FETUS	TOOTH	MOIST WEIGHT	MOISTURE[2] (MOIST WEIGHT)	INORG. (DRY WGT.)	Ca (INORG. WGT.)	Mg (INORG. WGT.)	P (INORG. WGT.(	RESIDUE SOLUTION NUMBERS	$\frac{Ca}{P}$
		gm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
E	a|a	0.1054	9.11	85.2	35.4		16.7	14.0	2.12
	b|b	0.0648	7.56	84.8	35.4		16.8	12.9	2.11
	c|c	0.0224	4.02	84.7	35.7		16.8		2.12
	d|d	0.0492	6.10	83.4	35.8		16.8	12.7	2.13
	a|a	0.0250	6.40	87.6	35.7		16.7	12.7	2.14
	b|b	0.0348	4.31	84.4	35.4		16.9	12.9	2.09
F	a|a	0.1150	10.40		33.3		16.9	12.4	1.97
	b|b	0.0636	8.65	79.2	34.0		17.2	11.3	1.98
	c|c	0.0237	5.91	78.5	33.7		16.8	12.1	2.00
	d|d	0.0979	7.46		33.1		16.9	12.8	1.96
	e|e	0.0632	6.49	67.7*	33.4		16.4	12.8	2.04
	a|a	0.0530	6.04	92.1*	33.1		16.7	14.1	1.98
	b|b	0.0400	5.00	91.6	32.3		16.7	12.6	1.93
	c|c	0.0182	3.85	76.6	33.7		17.1	14.9	1.97
	d|d	0.0771	5.71	76.2	34.0		16.6	12.1	2.05
	e|e	0.0497	4.02	73.8	34.4		16.7	13.3	2.06

Part 2

	MOISTURE[2] IN MOIST WEIGHT	INORG. (DRY WGT.)	Ca (INORG. WGT.)	Mg (INORG. WGT.)	P (INORG. WGT.)	RESIDUE SOLUTION NUMBERS	$\frac{Ca}{P}$	CO_3
	per cent	*per cent*	*per cent*	*per cent*	*per cent*			*per cent*
Average	7.09	80.4	34.2	1.12	16.8	13.8	2.03	3.30
Mean deviation	1.46	3.19	1.23	0.47	0.31	1.76	0.09	0.09
Standard deviation	2.0	4.5	2.3	0.6	0.5	2.4	0.2	0.1
Average on basis of s. d.	6.82	80.4	34.5	1.12	16.9	13.4	2.06	3.30
Number of atypical values	1	2	1	0	1	3	2	0
Number of typical values	58	51	54	12	54	52	51	13

Footnotes to Table I

Part 1

* Values discarded on the basis of standard deviation.

[1] The values for per cent Ca, P, and Mg, and residue solution number, represent averages of duplicate determinations.

[2] The term "moisture" is here used advisedly in preference to "water" since it is recognised that part of the water content of the tooth substance is held as "water of crystallisation" or "water of constitution" and is not driven off at 100°C. (Gabriel, (4)).

TABLE II

SOURCE	MATERIAL	CO_3 (INORG. WGT.)
		per cent
Term fetus	Single tooth	3.46
	" "	3.17
	" "	3.23
	" "	3.17
Term fetus	All teeth pooled	
	Average of seven determinations	3.29
Seven-month fetus	All teeth pooled	
	Average of two determinations	3.40

reported by Edwards (8), who states that adult bones have a greater carbonate content than children's bones and that healthily developed bones contain more carbonate than newly calcified bone at the site of

TABLE III

Relation of moisture percentage to moist weight of fetal teeth

FETUS	TEETH	MOIST WEIGHT	MOISTURE
		gm.	*per cent*
L	d|d	0.1468	19.3
L	a|a	0.1459	15.3
K	d|d	0.1201	19.6
L	d|d	0.1170	17.1
L	e|e	0.1122	10.1
L	b|b	0.1055	14.0
K	a|a	0.1054	18.0
K	d|d	0.0892	15.3
L	e|e	0.0880	13.4
L	a|a	0.0779	12.1
K	b|b	0.0762	18.1
M	d|d	0.0750	14.1
L	b|b	0.0633	15.0
M	b|b	0.0607	14.7
K	e|e	0.0590	12.2
K	a|a	0.0510	12.2
M	d|d	0.0502	11.8
M	a|a	0.0401	9.98
M	a|a	0.0374	10.2
K	e|e	0.0372	9.68
K	b|b	0.0357	12.0
M	e|e	0.0355	9.03
M	e|e	0.0347	8.66
L	c|c	0.0316	8.45
L	c|c	0.0285	8.77
M	b|b	0.0260	7.69
K	c|c	0.0218	9.18
K	c|c	0.0172	15.3
M	c|c	0.0163	9.21
M	c|c	0.0147	8.84

a fracture. Kramer and Shear (9) show that the carbonate content of rat bones increases with age and also that old bone contains more carbonate than young. In view of these facts a lower carbonate con-

tent reported for fetal teeth seems logical since they can be considered as newly calcified as compared to fully erupted deciduous or permanent teeth.

Fourthly, fetal teeth are reported to have a smaller calcium content than permanent teeth. Since amounts of inorganic tooth constituents are expressed in the literature in many different ways, e.g. as per cent of moist weight, per cent of ash weight, and so forth, the Ca:P per cent ratio is used as a basis for comparison of the data; for inasmuch as the phosphorus content of bone and tooth substance is known to be fairly constant, variation in calcium content is expressed in the Ca:P per cent ratio. Accordingly—reports in the literature show for infant teeth Ca:P per cent ratios of 2.26 (Hoppe-Seyler), and

TABLE IV

Analysis of fetal teeth using gravimetric methods

FETUS	JAW	Ca	P	$\frac{Ca}{P}$
		per cent	*per cent*	
G	Both	36.3	17.3	2.10
H	Upper	36.3	17.3	2.10
	Lower	36.5	17.4	2.10
I	Both	36.6	17.7	2.07
K	Both	36.4	17.3	2.10
L	Upper	36.5	17.2	2.12
	Lower	36.4	17.4	2.09
M	Both	36.5	17.3	2.11

2.04 (Hess, Lewis, and Roman). The average Ca:P per cent ratio found in this investigation is 2.04. To obtain Ca:P per cent ratios for fetal teeth using standard gravimetric methods (26) now employed by us for adult teeth, the pooled teeth from six additional term fetuses were analysed for calcium and phosphorus (Table 4). The Ca:P per cent ratios obtained average 2.10. Ratios reported for permanent teeth (4) are higher: Pepys (1814)—2.13; Lassaigne (1821)—2.29; Gabriel (1895)—dentine, 2.14—enamel, 2.14; Gassman (1908)—2.26; and Crowell et al. (1934)—2.31.

The literature concerning the inorganic composition of fetal bone substance shows values for inorganic constituents similar to those for fetal teeth. Von Recklinghausen (10) (1858) found values for the head bones of a term fetus corresponding to a Ca:P per cent ratio

of 2.10, in agreement with a ratio of 2.04 found by Brubacher (11) (1890), who analyzed the femur of a seven-month old fetus. In 1931, Yoshida (12) related an increase in calcium and a decrease in phosphorus in the parietal bones of a fetus to advance in age from fourth to tenth month of fetal life, his reports show a range in Ca:P per cent ratios from 2.34 at the fourth month to 4.42 at the tenth month. The average value for magnesium of 0.953% (Brubacher) in the femur of a seven-month fetus agrees with that from this investigation of fetal teeth of 1.12. The above data indicate that, from the standpoint of percentage composition, the inorganic substances of fetal tooth and fetal bone are similar.

The difference in Ca:P per cent ratio between fetal and adult tooth substance raises the question whether this is evidence of a difference in molecular constitution of the inorganic substance. There are several experimental facts in the literature indicating such a difference. Crystallographic studies by polarized light (13) have shown that the "matured" or erupted portion of a rat incisor differs from that just erupting, and that both differ from the most recently calcified portions. Hardness studies (14) have shown deciduous tooth substance to differ from permanent. The staining properties (15) of freshly erupted teeth differ from those of "matured" teeth. Several investigators (See reference 3, p. 261) have shown chemical changes to occur with age in teeth. Brooke, Smith and Smith (16) say mildly that "strictly chemical methods of approach have not yielded unequivocal results" in interpreting the bearing of analytical data on the molecular nature of the inorganic portion of bone or tooth substance. The justice of their statement is well born out in the following calculations of molecular constitution from analytical data:

1. Assume tooth substance is $Ca_3(PO_4)_2$ and $CaCO_3$ and something?

Fetal		*Adult* (3)	
% P = 16.8 = 84.00% $Ca_3(PO_4)_2$		% P = 16.3 = 82.00% $Ca_3(PO_4)_2$	
% CO_2 = 3.3 = 7.48% $CaCO_3$		% CO_2 = 4(approx) = 9.1% $CaCO_3$	
84.00% $Ca_3(PO_4)_2$	= 32.6% Ca	82.00% $Ca_3(PO_4)_2$	= 31.8% Ca
7.48% $CaCO_3$	= 3.00	9.1% $CaCO_3$	= 3.6% Ca
Required by PO_4 & CO_3	= 35.6%	Required by PO_4 & CO_3	= 35.4% Ca
% Ca Found	= 34.5	% Ca Found	= 37.5
Therefore, Ca deficit.		Therefore, Ca excess.	

In adult teeth the per cent Ca found being greater than the per cent Ca needed to react with the PO_4 and CO_3 the "basic excess" (4) is assumed to be OH. A "basic excess"

is also found for bone. In fetal teeth the per cent Ca found is insufficient to account for the PO_4 and CO_3 present; thus, having a Ca deficit, we might logically assume fetal teeth to be different in molecular make up from adult.

Morgulis first employed the term "basic excess" to express the number of liters of N HCl necessary just to dissolve 1000 grams of inorganic residue. Since in adult tooth substance the number of liters of N HCl is always greater than the number needed to replace the PO_4 and CO_3 as determined by analysis, there must exist an excess of basic substances, i.e., Ca and Mg. This "basic excess," being of the magnitude of 4–6% of the total inorganic substance, could not be accounted for by fluoride, chloride, or sulphate; consequently Morgulis assumed for bone that the "basic excess" is combined with OH. This assumption seemed the more plausible from the repeated demonstrations by roentgen-ray studies that an OH-apatite is the principal constituent of bone substance (17, 18). Crowell et al. offer a francolite-like formula, $3Ca_3(PO_4)_2 \cdot CaCO_3 \cdot Ca(OH)_2$, for adult tooth substance on a similar basis. However, in the case of fetal tooth substance, where the Ca content is slightly less than that needed to react with the PO_4 and CO_3 present, the concept of a "basic excess" is not valid. Still, the number of liters of N HCl necessary to dissolve 1000 grams of inorganic residue may be experimentally determined for fetal teeth. We believe that the more generally applicable term for this figure is "residue solution number" and consequently use it to describe the data found. (Table I.)

2. Assume tooth substance is a mixture of $Ca_3(PO_4)_2$ and $CaCO_3$.

Fetal		*Adult* (3)	
Total Ca	= 34.5%	Total Ca	= 37.5%
Ca as CO_3	= 3.00%	Ca as CO_3	= 3.6%
Residual Ca	= 31.5%	Residual Ca	= 33.9%
Residual Ca	= 0.787 mols/100 gm.	Residual Ca	= 0.848 mols/100 gm.
P	= 16.8%	P	= 16.3%
P	= 0.542 mols/100 gm.	P	= 0.526 mols/100 gm.
$\frac{\text{Residual Ca (mols)}}{\text{P (mols)}}$		$\frac{\text{Residual Ca (mols)}}{\text{P (mols)}}$	
	$= \frac{0.787}{0.542} = 1.45$		$= \frac{0.848}{0.526} = 1.61$

If we compute the amount of Ca combined with CO_3 and subtract this quantity from the total Ca, it is interesting that in the adult tooth the residual Ca (mols) is to the P (mols) nearly as Ca is to P in $Ca_3(PO_4)_2$, i.e., 1.5. In fetal teeth, the residual Ca to P mol ratio is even closer to that required by $Ca_3(PO_4)_2$. We may argue from this evidence that fetal and adult tooth substances are much the same. Bone (19) also has a residual Ca to P mol ratio close to 1.5. This fact, together with certain roentgen-ray studies (20), has been recently used to support the assumption that bone is fundamentally a mixture of $Ca_3(PO_4)_2$ and $CaCO_3$.

3. Assume that tooth substance is $CaCO_3 \cdot n\ Ca_3(PO_4)_2$:

Fetal		*Adult* (3)	
CO_2	= 3.3%	CO_2	= 4%
$CaCO_3$ from CO_2	= 7.48%	$CaCO_3$ from CO_2	= 9.1%
$CaCO_3$ from CO_2	= 0.075 mols/100 gm.	$CaCO_3$ from CO_2	= 0.091 mols/100 gm.
Residual Ca	= 31.5%	Residual Ca	= 33.9%
$Ca_3(PO_4)_2$ from residual Ca	= 81.2%	$Ca_3(PO_4)_2$ from residual Ca	= 87.3%

Fetal	*Adult* (3)
$Ca_3(PO_4)_2$ from residual Ca = 0.262 mols/100 gm.	$Ca_3(PO_4)_2$ from residual Ca = 0.281 mols/100 gm.
$\frac{Ca_3(PO_4)_2 \text{ from residual Ca (mols)}}{CaCO_3 \text{ (mols)}} = \frac{0.262}{0.075} = 3.50$	$\frac{Ca_3(PO_4)_2 \text{ from residual Ca (mols)}}{Ca_3 \text{ (mols)}} = \frac{0.281}{0.091} = 3.09$
Therefore $CaCO_3 \cdot 3.50\ Ca_3(PO_4)_2$	Therefore $CaCO_3 \cdot 3.09\ Ca_3(PO_4)_2$
P = 16.8%	P = 16.3%
$Ca_3(PO_4)_2$ from P = 84.00%	$Ca_3(PO_4)_2$ from P = 82.00%
$Ca_3(PO_4)_2$ from P = 0.271 mols/100 gm.	$Ca_3(PO_4)_2$ from P = 0.264 mols/100 gm.
$\frac{Ca_3(PO_4)_2 \text{ from P (mols)}}{CaCO_3 \text{ (mols)}} = \frac{0.271}{0.075} = 3.61$	$\frac{Ca_3(PO_4)_2 \text{ from P (mols)}}{CaCO_3 \text{ (mols)}} = \frac{0.264}{0.091} = 2.90$
Therefore $CaCO_3 \cdot 3.61\ Ca_3(PO_4)_2$	Therefore $CaCO_3 \cdot 2.90\ Ca_3(PO_4)_2$

If tooth substance is CO_3-apatite, the formula calls for three molecules of $Ca_3(PO_4)_2$ to one of $CaCO_3$. It has been computed (21) that adult bone has between two and three molecules of $Ca_3(PO_4)_2$ to one of $CaCO_3$. For adult teeth, if we calculate this ration from the residual Ca, we find n, the number of mols of $Ca_3(PO_4)_2$, equals 3.09; calculating from the P content, n is 2.90. These values indicate a close correspondence of adult tooth substance to CO_3-apatite. In the case of fetal teeth, calculating from the residual Ca, n is 3.50; from the P content, n is 3.61. Since neither of these values closely approximates the formula for CO_3-apatite, we might argue that fetal and adult tooth substance are fundamentally different.

Collophane, whose formula, $3\ Ca_3(PO_4)_2 \cdot n\ CaX_2 \cdot XH_2O$ where $1<n<2$ and X is variable, is regarded by Rogers (25) as the amorphous equivalent of the apatite, dahllite, $3\ Ca_3(PO_4)_2 \cdot CaCO_3$. Calculating on this basis, for fetal tooth substance n is about 0.9, X not known, for adult tooth substance n is about 1.0, X about 0.3.

4. Assume tooth substance is $Ca(OH)_2 \cdot Ca_3(PO_4)_2$:

Fetal		*Adult*	
Total Ca	= 34.5%	Total Ca	= 35.4%
P	= 16.8%	P	= 16.7%
$\frac{Ca\%}{P\%}$	= 2.04	$\frac{Ca\%}{P\%}$	= 2.12 (unpublished data of the authors)
$Ca(OH)_2 \cdot 6\ Ca_3(PO_4)_2$ has $\frac{Ca\%}{P}$	= 2.04	$Ca(OH)_2 \cdot 3\ Ca_3(PO_4)_2$ has $\frac{Ca\%}{P}$	= 2.15

If we assume that tooth substance is fundamentally OH-apatite, composed of one molecule of $Ca(OH)_2$ with three molecules of $Ca_3(PO_4)_2$, the Ca:P per cent ratio is theoretically 2.15. For adult tooth substance we find a Ca:P per cent ratio of 2.15 which corresponds well with the value found when n = 3. Furthermore, the "basic excess" shown to exist may well be explained on the basis of an OH compound. On the other hand, the fetal tooth substance has a Ca:P per cent ratio of 2.04 which corresponds to Morgulis' proposed empirical formula for bone, in which n = 6.

5. Assume tooth substance is of the apatite series:

Fetal		*Adult* (3)	
Residue Solution Number	= 13.4	Residue Solution Number	= 12.7
For 6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$	= 13.45	For $Ca_3(PO_4)_2$	= 12.9

Assuming tooth substance to be a mineral of the apatite series and comparing the residue solution numbers of the fetal and adult tooth substance with residue solution numbers of various apatites, we find a correspondence between 6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$ and fetal teeth and between $Ca_3(PO_4)_2$ and adult teeth. From Morgulis' "basic excess" figures, bone is oftener like $Ca_3(PO_4)_2$ than like 6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$.

On the assumption that there may be a mixture of apatites, for example, part CO_3-apatite and part HPO_4-apatite (24), the adult tooth substance might be considered to be pure CO_3-apatite as far as the CO_2 per cent and the Ca:P per cent ratio are concerned. The fetal tooth substance might be described as a mixture of 77% CO_3- and 23% HPO_4-apatites on the same bases. Neither of these formulations gives close agreement with the actual percentages of Ca and P found.

6. Assume adsorption of PO_4 on fundamental crystal:

It seems reasonable to assume that the *fundamental* substance of both fetal and adult teeth may be the same chemical compound. By analysis, however, it is evident that the fetal and adult teeth differ principally in the respective Ca per cents and Ca:P per cent ratios. We may explain this difference in the chemical composition by assuming that in the fetal tooth, more PO_4 is adsorbed (22) on the large surface of the many tiny crystals, thereby decreasing the Ca per cent and the Ca:P per cent ratio.

In summary, adult and fetal tooth substance may be assumed to be different: 1) from the Ca excess of the adult and the Ca deficit of the fetal tooth substance; 2) from the calculated mol ratios of $Ca_3(PO_4)_2$ to $CaCO_3$; 3) from the Ca:P per cent ratio; 4) from the residue solution numbers; and 5) from the CO_2 per cents. On the other hand, adult and fetal tooth substance may be assumed to be the same: 1) from the mol ratios of residual Ca to P, if tooth substance is a mixture of $Ca_3(PO_4)_2$ and $CaCO_3$; and 2) if the difference in Ca per cents and Ca:P per cent ratios is due to an adsorption of PO_4 on the minute crystals of the fundamental substance.

X-RAY DIFFRACTION PATTERNS

Using x-ray diffraction methods, a number of investigators (see bibliography of reference 23) have shown that the principal constituent of adult tooth substance is a crystalline substance closely similar to the mineral apatite; and also, by Bale et al., that the crystalline particles are exceedingly minute, probably not exceeding 10^{-6} cm. mean size in the dentine (23). Supplementing the chemical analysis of fetal teeth, x-ray powder spectroscopy studies were made of tooth samples C a|a, C d|d, C d|d, D a|a, D d|d, F a|a, and F d|d, of sample Z

(human enamel), sample X (adult tooth substance), sample Y (deciduous tooth substance), and 14 samples of fluor- and chlor-apatites from all parts of the world. The General Electric x-ray diffraction apparatus type YWC, form D (1) was used with the regulation diffraction cassettes provided. With these cassettes, two diffraction photographs are made, side by side, on the same film under identical conditions, thus greatly facilitating the comparison of the relative intensities as well as the line positions of two different substances.

In Figure II, (A) is the x-ray powder diffraction spectrum of apatite mineral $Ca_{10}F_2(PO_4)_6$; (B) is the diffraction spectrum of sample Z adult tooth substance (a mixture of enamel, dentine and cementum);

Fig. 2. X-ray diffraction patterns of (A) fluor-apatite, (B) adult human tooth substance, (C) adult human enamel, (D) fetal tooth substance. Note that the (004) diffraction maximum at X is more intense in tooth patterns than in apatite; also that lines at Y and Z in the apatite pattern are fainter in tooth substance patterns.

(C) is the diffraction spectrum of sample X adult human enamel; and (D) is the diffraction spectrum of fetal tooth sample D a|a. All four diffraction films are essentially similar. These diffraction photographs are confirmatory evidence that tooth substance, adult and fetal, is composed mainly of a mineral constituent similar to the natural mineral apatite. Since crystalline calcium carbonate gives diffraction maxima entirely dissimilar to the tooth patterns shown, it should be possible to recognize the presence in tooth substance of 3–4 per cent calcium carbonate. There is no indication of its presence.

All three of the diffraction patterns of tooth substance, i.e., *Fig. 2*, (B), (C) and (D), appear exactly alike as far as line positions and intensities are concerned. The adult and fetal tooth substance photo-

graphs have a greater diffuseness of diffraction maxima than has enamel, indicating that the dentine mineral crystals are smaller than those in enamel and that the crystalline particles in fetal tooth substance are about the same order of magnitude as those in adult tooth substance. The other fetal teeth studied gave diffraction patterns which were, in every respect, similar to the example in *Fig. 2*. A close examination of the typical apatite diffraction pattern (A) shows that there are certain differences from the tooth substance diffraction pattern, especially in the lines corresponding to higher Miller indices. Thus, the (004) diffraction maximum noted as X, *Fig. 2*, is relatively more intense in the tooth substance than in the apatite pattern. Also, at Bragg angles greater than that of (004) other consistent differences occur; notably at Y and Z, *Fig. 2*, there are lines prominent in the apatite pattern, but much less intense in the diffraction patterns of tooth substance.

X-ray diffraction evidence, then, indicates that the crystal structure of the principal mineral constituent of fetal and adult tooth substance is similar, and that it belongs to the apatite series of minerals. In the absence of conclusive evidence, the principal constituent may be represented as comparable to a fluor-apatite in which F has been replaced by either CO_3 or OH, or both.

It is interesting to inquire what changes in chemical composition are compatible with the constancy of diffraction patterns here shown; i.e., would the compound 6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$ give a diffraction pattern identical with that of 3 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$. If identity of diffraction patterns means identity of fundamental tooth substance, fetal and adult, there are two chemical possibilities given above: either they are both mixtures of $Ca_3(PO_4)_2$ and $CaCO_3$, or the chemical differences are to be explained by adsorption of PO_4. Since the x-ray diffraction patterns exclude the presence of more than traces of $CaCO_3$, the only valid assumption remaining is that adsorption of PO_4 on the minute apatite crystals results in a difference of chemical composition. Is this fundamental apatite $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$ and, if so, what of the CO_3 content?

SUMMARY

1. Normal values for fetal tooth substances are as follows: (per cent) moisture, 6.81 ± 2.0; inorganic residue, 80.4 ± 3.2; Ca, 34.5

$\pm$ 2.3; Mg, 1.1 $\pm$ 0.6; P, 16.9 $\pm$ 0.5; CO_2, 3.3 $\pm$ 0.1; residue solution number, 13.4 $\pm$ 2.4; Ca:P per cent ratio, 2.04 $\pm$ 0.16.

2. Per cents of Ca, P, inorganic residue are constant and per cents of Mg, moisture and residue solution numbers are variable.

3. Central incisors, upper and lower, have a higher inorganic residue than other teeth; otherwise, there is no relation between tooth type and per cent composition.

4. There is a definite relation between the moist weight of the fetal tooth and its morphological type.

5. Ca per cent, Ca:P per cent ratio and moisture per cent are lower for fetal than for adult teeth.

6. It is impossible to state on the basis of chemical analysis whether fetal and adult tooth substance have the same molecular structure since certain calculations show similarities in constitution and others show differences.

7. X-ray diffraction patterns for fetal and adult tooth substance are identical; this is strong presumptive evidence that the principal constituents are identical.

REFERENCES TO LITERATURE

(1) HOPPE-SEYLER, F. *Virchow's Archiv 24:* 13, 1862.
(2) HESS, A. F., LEWIS, J. M., AND ROMAN, B. *Den. Cosmos 74:* 1053, 1932.
(3) CROWELL, C. D., HODGE, H. C., AND LINE, W. R. *J. Den. Res. 14:* 251, 1934.
(4) See bibliography of *reference 3*, numbers (3), (15), (18), (17) and (16).
(5) SHEAR, M. J. AND KRAMER, B. *J. Biol. Chem. 79:* 105, 1928.
(6) HOPEWELL-SMITH, A. "Deutal Anatomy and Physiology." Lea and Febiger, Philadelphia, 1913, p. 74.
(7) BOOHER, L. E. AND HANSMANN, G. H. *J. Biol. Chem. 94:* 195, 1931.
(8) EDWARDS, A. M. *Ann. des Sci. Nat.*, ser. 4, *13:* 113, 1860.
(9) KRAMER, B. AND SHEAR, M. J. *J. Biol. Chem. 79:* 147, 1928.
(10) VON RECKLINGHAUSEN, F. *Virchow's Archiv 14:* 466, 1858.
(11) BRUBACHER, H. *Zeit. Biol. 27:* 517, 1890.
(12) YOSHIDA, I. Arbeiten Medizinishe Universität. *Okayama 2:* 326, 1931.
(13) CAPE, A. T. AND KITCHIN, P. C. *J. Am. Den. Assoc. 17:* 193, 1930.
(14) KARLSTRÖM, S. "Physical, Physiological, and Pathological Studies of the Dental Enamel," Fahlcrantz, Stockholm, 1931.
(15) BEUST, T. B. *J. Am. Den. Assoc. 18:* 2186, 1931.
(16) BROOKE, R. O., SMITH, A. H., AND SMITH, P. K. *J. Biol. Chem. 104:* 141, 1934.
(17) BREDIG, M. A. *Zeit. Physiol. Chem. 216:* 239, 1933.
(18) MÖLLER, H. AND TRÖMEL, G. *Naturwiss. 21:* 346, 1933.
(19) MAREK, J., WELLMANN, O., AND URBANYI, L. *Zeit. Physiol. Chem. 226:* 3, 1934.

(20) FUNAOKA, S. *Ber. Physiol. 63:* 388, 1932.
(21) ROSEBERRY, H. H., HASTINGS, A. B., AND MORSE, J. K. *J. Biol. Chem. 90:* 395, 1931.
(22) CAMERON, F. K. AND BELL, J. M. *J. Am. Chem. Soc. 27:* 1512, 1905.
(23) BALE, W. F., HODGE, H. C., AND WARREN, S. L. *Am. J. Roentg. 32:* 369, 1934.
(24) BUCH. *Zeit. anorg. Chem. 52:* 325, 1920.
(25) ROGERS. *Bull. Geol. Soc. Amer. 35:* 535, 1923.
(26) WASHBURN, M. L., AND SHEAR, M. J. *J. Biol. Chem. 99:* 21, 1932

GROWTH OF THE PALATE IN THE HUMAN FETUS

BENJAMIN FREIBAND, M.S.

Department of Anatomy, College of Dentistry, New York University and Department of Histology, School of Medicine, American University of Beirut, Syria

INTRODUCTION

Human dental anatomy was established on a basis of careful and detailed, naked-eye observation in the middle of the sixteenth century. It is unnecessary here to detail the work of the investigators of that time. It may be recalled that Eustachius, (3) in 1563, published the first books entirely devoted to anatomy and development of the human teeth, jaws, and arches, and that this work corrected many errors of ancients and contemporaries. One of the problems which caused much discussion among dental scientists during the years to follow, related to the site of development of bone in the growth of jaws and dental arches. John Hunter (5) apparently was responsible for the earliest hypothesis, that the permanent teeth have no more space for their accommodation than had their predecessors. Dental investigators and practitioners were immediately divided, being either proponents of Hunter's proposition or believing that there was horizontal growth in the anterior segment of the jaws as well as back of the second bicuspid. Neither Hunter nor his immediate successors on either side of the controversy furnished convincing observational evidence. In 1824, Oudet (7) presented before the Académie Royale de Medicine, results of a study of impressions of the arches of 20 children, 3 years of age and older, agreeing with Hunter. But in the developmental field, these early workers had of necessity left much to be desired. In more recent years although extensive investigations have been conducted on the postnatal development of jaws and palate, knowledge of the fetal palate remains inadequate. A more detailed concept of prenatal growth is important as a basis for interpretation of palate types and arch forms in postnatal life. This paper is an attempt to fill this gap in the knowledge of the growth and development of the human palate.

LITERATURE

Few studies are available on quantitative growth of the fetal palate. John Hunter (5) in the first good account of teeth, jaws and palatal arches of the fetus stated,

"In the Foetus three or four months old, we have described the marks of four or five teeth, which occupy the whole length of the Upper Jaw, and all that part of the Lower which lies before the Coronoid Process, for the fifth Tooth is somewhat under that process. These five marks become larger, and the Jawbones of course increase in all directions, but more considerably backwards; for in a Foetus of seven or eight months, the marks of six Teeth on each side of both Jaws are to be observed, and the sixth seems to be in place where the fifth was; so that in these last four months the Jaw has grown in all directions, in proportion to the increased size of the Teeth, and besides has lengthened itself at its posterior end as much as the whole breadth of the socket of that sixth Tooth. The Jaw still increases in all points till twelve months after birth, when the bodies of all the sixth teeth are pretty well formed; but it never after increases in length between the symphysis and the sixth Tooth; and from this time, too, the Alveolar Process, which marks the anterior part of the child's face, is flatter, or not so projecting forwards as in the adult. After this time the Jaws lengthen only at their posterior ends."

Alkan (1) measured with the aid of calipers, in the newborn and infant palate, the length from central incisors to the end of the hard palate and the width across the lingual margins at about the middle of the alveolar ridges. He constructed a triangle with base between points taken at the middle of the alveolar margins, and apex approximately at the center of the palate arch, and calculated the height as the altitude of the triangle. He concluded that the newborn palate is rather short and high, but did not refer to growth.

Franke (4) studied a small number of macerated upper maxillas of fetuses, ranging in age from five months to birth. His measurements appear to lack significance for quantitative studies of palate form, since he was primarily interested in relationships of the developing deciduous teeth to the tooth sacs, and the development of deformed jaws and nasal septa. Katz (6) investigated the dimensions of the fetal palate and the origin of palate form and arrived at the following conclusions[1]:

"The hard palate of the fetus, that which lies between the external alveolar

---

[1] Translated from the unpublished original manuscript in the library of the Senchenbergische Institut Anatomie, Universitat Frankfurt a.M.

groove from the alveolar wall and the separation through a small inflection of the oral cavity of the soft palate, shows more frequently the horseshoe form and an intermediate form to the U-form. The perfect U-form is rare, more rare than the parabolic form that the adult frequently exhibits. In some fetal palate forms one may easily perceive the early form of the later deformities, and to all appearance in the later embryonic life the particular palate form of the adult *in statu nascendi*. In respect of breadth, height, and length, the fetal forms vary considerably. At this stage one can already speak of broad, long and high palate types. The fetal palate compared to the adult is flatter and broader, because the alveolar tooth carrying process is absent and because the roof of the oral cavity has the tendency to develop more posteriorly than laterally. The question of the origin of the palate form appears, in spite of the investigations of various authors, still open."

West (9) observed that the gum ridges in the human fetus are segmented to correspond directly in relation to the tooth sacs below. Embryologically, the alveolar mucosa develop from two distinct parts, labio-buccal and lingual. The labio-buccal portion differentiates first and grows more rapidly, while the lingual portion differentiates later and remains almost entirely smooth. Concerning the segmentation of these tissues overlying the hard palate, West stated:

"In the account of the development of the gums it was stated that one of the ways in which the two portions differ is that the labio-buccal portion is divided into a number of segments, whereas the lingual portion is not. The gum at first has a smooth and regular outline, but in a fetus of 90 mm. it can be seen that the labio-buccal portion is becoming divided into a number of segments by means of a series of vertical grooves. The segmentation increases as development proceeds, being most noticeable about the eighth month, and at this stage it may be so well marked as to simulate newly erupted teeth. The number of segments is always regular and corresponds to the number of deciduous teeth, to which, as will subsequently be shown, they are intimately related. There are, therefore, five segments in each half of each jaw, which may be termed, in correspondence with the deciduous teeth, medial and lateral incisor, canine, and first and second molar segments. The medial incisor and canine segments are about equal in size, the lateral incisor a good deal smaller, and the first molar is the largest. The second molar, owing to the fact that the labio-buccal portion of the gum becomes progressively smaller as it is traced backwards, is frequently difficult to recognize at all."

The various segments of the gum observed by West are shown in his Plate I.

Ashley-Montagu (2) studied the form and dimensions of the palate from a series of casts of 90 newborn children. He used a more exact method of measurement, and included the sulci and points corresponding to the underlying bony structures. From computed re-

sults on the palatal index, he found the type palate of the newborn to be brachyuranic. He noted that average anterior palatal breadth is equal to average posterior palatal breadth, and that each of these is nearly equal to maximum palatal length. The palatal height is 7.6 ± 2.0 mm. and is usually found at the center of the palate.

MATERIAL AND METHOD

The study is based on a series of palate casts from 102 fetuses, aged from 3.2 fetal months to birth, obtained from the New York City mortuary. Body measurements were taken on these specimens and the fetal age calculated from the crown-heel length by the use of the formula of Scammon and Calkins (8):

$$T = 2.3 + \frac{2.5L}{28} + \frac{L2}{784}$$

where T is calculated fetal age, and L is total body length (CH). Since information concerning race and nationality of these specimens was inadequate, special grouping was deemed inadvisable. No pathological material was used. Stone casts of the palates were made from plaster impressions taken soon after death. These casts were exact reproductions of the fleshy palates, showing all the details desired. Furthermore, measurements of them were found to be far more accurate than those obtainable on the individual fetuses *in situ*.

Certain landmarks observed on the fleshy palate have been described in the fetus (2), (9), corresponding with grooves and boundaries of the underlying bony structures. The morphological divisions of the palate or alveoli are shown in *Fig. 1*, a pantograph tracing of a palate cast. On the gingival ridge, A, the site of eruption of the deciduous teeth, are located certain points from which the sulci take origin. The post-gingivale, 1, represents the posterior limitation of the lingual portion of the gum. The molon point, 2, is the lowermost projection of the gingival ridge. The lateral sulcus point, 3, the antero-lateral sulcus point, 4, the anterior sulcus point, 5, and the incisive point, 6, complete the surface markings on this ridge.

The dental and lingual grooves are CC and DD, respectively. The labio-buccal division of the gum is bounded by the gingival ridge, A, and the dental groove, CC. The lingual division of the gum is situated between the dental groove, CC, and the lingual groove, DD. The outer buccal margin, B, describes the lateral limit of the superior maxilla. The sulci

corresponding to the crypt walls separating the deciduous teeth are observed on the labio-buccal portion of the gum and are five in number. Of these five, only the postero-lateral sulcus does not correspond to the underlying crypt wall and also extends across the lingual portion of the gum. The incisive groove, I, represents the separation between the medial incisors. It is continuous posteriorly with the incisve papilla,. J. The anterior sulcus, H, corresponds to the crypt wall separating the medial and lateral incisor tooth sacs. The antero-lateral sulcus, G, marks the

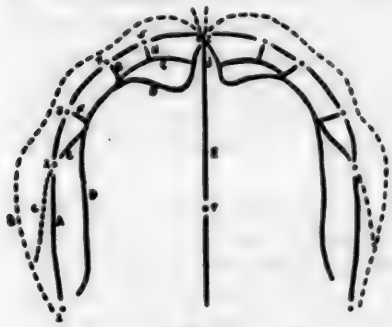

Fig. 1

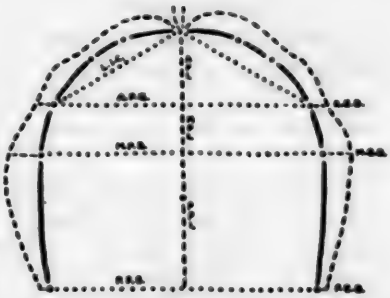

Fig. 2

Fig. 1. Pantograph representation of morphological divisions observed on fetal palate. A, gingival ridge; B, outer buccal margin; CC, dental groove; DD, lingual groove; E, median raphe; F, lateral sulcus; C, postero-lateral sulcus; G, antero-lateral sulcus; H, anterior sulcus; I, incisive groove; J, incisive papilla; 1, post-gingivale; 2, molon point; 3, lateral sulcus point; 4, antero-lateral sulcus point; 5, anterior sulcus point; 6, incisive point; 7, maximum palatal height.

Fig. 2. Pantograph tracing of fetal palate showing location of projection chords. A. P. B., anterior palatal breadth; M. P. B., maximum palatal breadth; P. P. B., posterior palatal breadth; A. P. L., anterior palatal length; M. P. L., middle palatal length; P. P. L., posterior palatal length; A. B. B., anterior buccal breadth; M. B. B., maximum buccal breadth; P. B. B., posterior buccal breadth; A. P. L. M. P. L. P. P. L., maximum palatal length.

boundary between the lateral incisor sac and the deciduous canine. The lateral sulcus, F, limits the deciduous canine anteriorly and the first deciduous molar posteriorly. The postero-lateral sulcus, C, more superficial than the others, extends across both lingual and labio-buccal portions of the palate, and represents the boundary between first and second deciduous molars. Other morphological landmarks on the fleshy palate are the median raphe, E, the incisive papilla, J, and several rugae extending transversely across the palatine portion of the soft tissue.

By using certain of these morphological landmarks, the palate can be oriented on an exact mathematical plane. Such a plane has been experimentally determined by Ashley-Montagu (2) on the newborn palate and can be used satisfactorily for the fetal palate. The landmarks for orientation are the incisive point, 6, and the two post-gingivale points, 1. The palate is oriented in this incisive-post-gingivale plane (palatine plane) on Stanton's Dental Pantograph, and the points, sulci and outlines drawn to a scale enlarged five times. The outer buccal margin of the palate is drawn and then the lowermost projection of the gum tissue representing the gingival ridge is recorded. Incisive point, anterior, antero-lateral, lateral and molon points are then registered on the drawing. Finally, incisive papilla, dental and lingual grooves are outlined; anterior, antero-lateral, lateral and postero-lateral sulci projected, and the height of the palate determined and its site registered by means of an elevation bar on the pantograph.

After these landmarks are recorded, the following chords are projected (*Fig. 2*): ***Maximum palatal breadth:***—greatest diameter between lowermost positions of the gingival ridge; ***maximum buccal breadth:***—greatest diameter between outer margins of the maxilla; ***anterior palatal breadth***—diameter between lateral sulcus points on the gingival ridge; ***anterior buccal breadth***—diameter between lateral sulcus points on the maxillary margin; ***posterior palatal breadth***—diameter between post-gingivale points on the gingival ridge; ***posterior buccal breadth***—diameter between post-gingivale points on the maxillary margin; ***maximum palatal length***—length from gingival incisive point to posterior breadth chord; ***maximum buccal length***—length from buccal incisive point to posterior breadth chord; ***anterior palatal length***—length from gingival incisive point to anterior breadth chord; ***middle palatal length***—distance on maximum length chord between anterior breadth chord and maximum breadth chord; ***posterior palatal length***—distance on maximum length chord between maximum breadth chord and posterior breadth chord; ***lateral incisive chords, right and left***—distances from gingival incisive point to lateral sulcus points; ***palatal height:***—the maximum height above the plane of gingival ridge.

These projection chords were then measured by means of a $\frac{1}{2}$ mm. scale, and the data obtained tabulated. In order to express quantitatively the growth tendency of the palate, it was desirable to convert the data into index values. Those used here are the palatal index, the anterior palatal index, and the palatal height index.

a. The ***palatal index*** expresses the relationship between the maximum length and the maximum breadth of the palate.

$$\text{Palatal index} = \frac{\text{Maximum breadth}}{\text{Maximum length}} \times 100$$

The results obtained from these indices are used in grouping palatal types in the fetus as follows:

Palate Types	*Index*
Dolichouranic	below 100.0–110.0
Mesuranic	110.1–120.0
Brachyuranic	120.1–130.0
Hyperbrachyuranic	130.1–140.0 or above

These palate types vary from the long and narrow dolichouranic form to the short and wide hyperbrachyuranic form as shown in the composite pantograph tracings in *Fig. 3.*

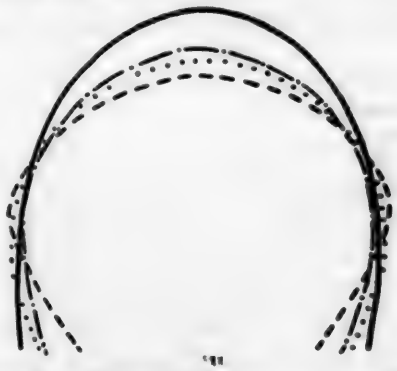

Fig. 3. Palate types observed during fetal period. ———, dolichouranic; .–.–.–.– mesuranic; ....., brachyuranic; ———, hyperbrachyuranic.

b. The *anterior palatal index* determines the anterior arch curvature, and is the ratio between anterior length and the anterior breadth of the palate.

$$\text{Anterior palatal index} = \frac{\text{Anterior length}}{\text{Anterior breadth}} \times 100$$

The values for anterior arch curvature are interpreted as follows:

Arch curvature	*Index*
Platyform	below 20.0–23.0
Mesiform	23.1–26.0
Cycloform	26.1–30.0
Propyriform	30.1–33.0
Pyriform	33.1–37.0 or above

c. The *palatal height index* expresses the relationship between height and maximum breadth of the palate.

$$\text{Palatal height index} = \frac{\text{Palatal height}}{\text{Maximum breadth}} \times 100$$

The index values obtained are used to plot several graphs, which serve to indicate the growth processes of the palate during fetal life. The findings are discussed below and the more important features considered in relation to (a) development of palate form; growth of the palate in (b) length; (c) breadth; and (d) height; (e) growth in length and breadth of the maxilla; and (f) relationship of palate growth to maxilla growth.

RESULTS

Earlier investigators of the fetal palate were concerned primarily with distinguishing the form and frequency of certain types of

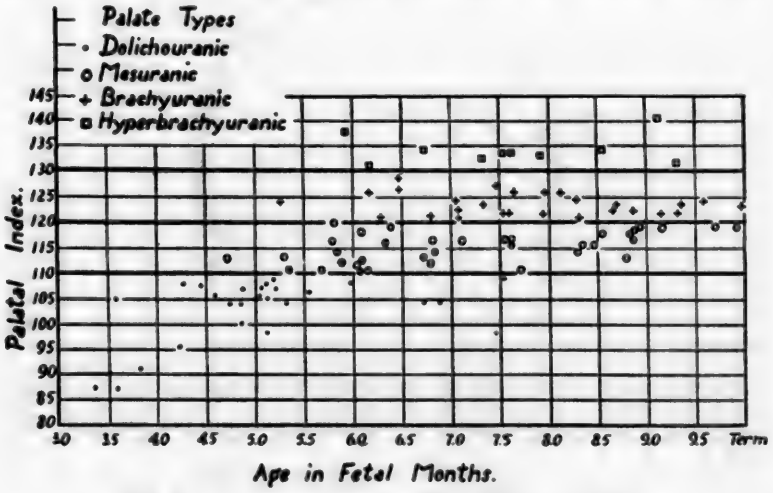

Fig. 4. Graph of palatal indices plotted against fetal age

palates, but neglected to associate palate form with possible developmental growth. They point out that the palate assumed various forms during fetal life, varying considerably in breadth, length and height. The mere existence of various types of palates in the fetus might indicate that after embryonic development a definite growth

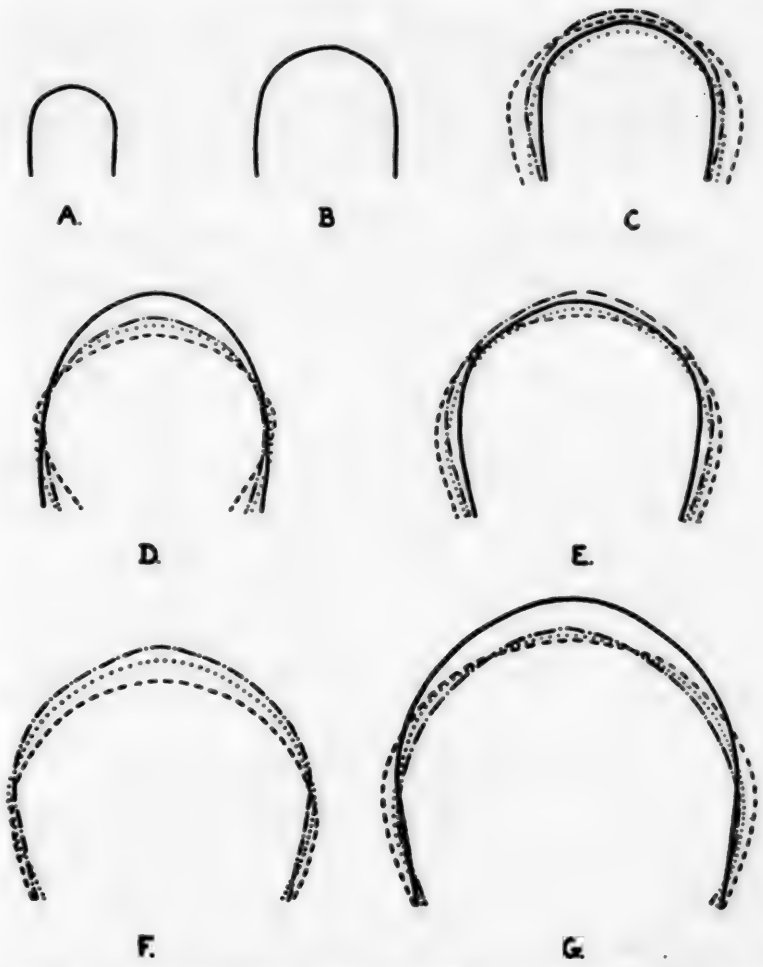

Fig. 5. Comparison of palate types observed during fetal growth period. A, 3–4 fetal months; B, 4–5 fetal months; C, 5–6 fetal months; D, 6–7 fetal months; E, 7–8 fetal months; F, 8–9 fetal months; G, 9 fetal months to term. ————, dolichouranic; .–.–.–.–, mesuranic; ....., brachyuranic; ————, hyperbrachyuranic.

process occurs, so that the palate form finally attained in the adult represents a series of growth stages. The findings given below bear out this assumption.

a. Development of palate form. In the course of examination of fetal palates, the frequent occurrence was noted of palate types similar to those seen in postnatal life. It appeared interesting to determine whether these fetal palate types were indications of progressive stages in development of adult palate form. From a study

TABLE I

Distribution of palate types and percentage values calculated for each fetal month

AGE FETAL MONTHS	NUMBER OF CASES	DOLICHOURANIC	MESURANIC	BRACHYURANIC	HYPERBRACHYURANIC
		4	—	—	—
3–4	4	100.0%	—	—	—
		8	1	—	—
4–5	9	87.5%	12.5%	—	—
		10	7	1	1
5–6	19	52.6%	36.8%	5.3%	5.3%
		2	11	5	2
6–7	20	10.0%	55.0%	25.0%	10.0%
		2	5	12	4
7–8	23	8.7%	21.7%	52.2%	17.4%
		—	9	6	1
8–9	16	—	56.2%	37.5%	6.3%
		1	3	5	2
9—Term	11	9.1%	27.2%	45.5%	18.2%

TABLE II

Distribution of palate types in relation to anterior arch curvature

PALATE TYPE	NUMBER OF CASES	PLATIFORM	MESIFORM	CYCLOFORM	PROPYRI-FORM	PYRIFORM
Dolichouranic	27	—	—	2	3	22
Mesuranic	36	—	—	11	16	9
Brachyuranic	29	—	2	12	8	7
Hyperbrachyuranic	10	2	2	5	1	—

of the grouping of palatal indices in *Fig. 4*, it appears that palate forms observed in early fetal months are of the dolichouranic type It is obvious that this type is carried over from the later embryonic period. In 4 cases aged from 3 to 4 fetal months all the palates were of the dolichouranic type, while in a series of fetuses from 4 to 5 months of age, 85 per cent show this characteristic form. Soon after

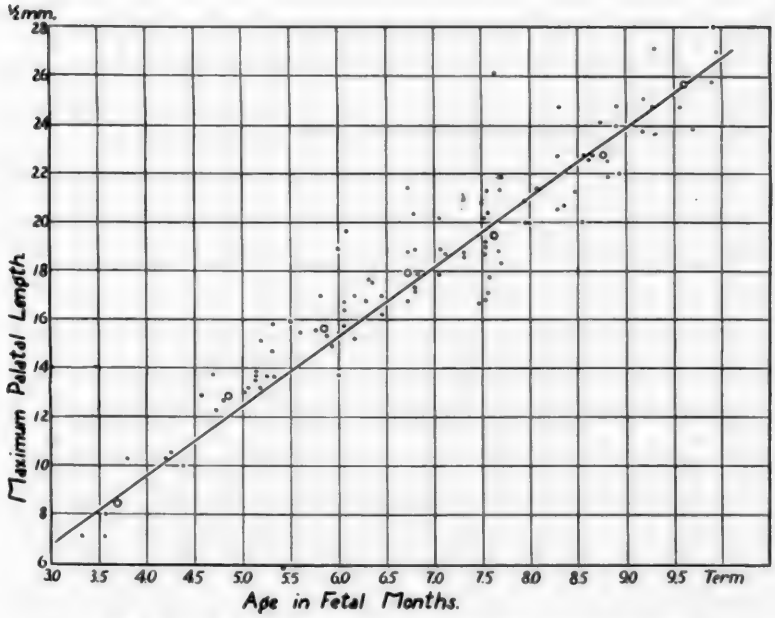

Fig. 6. Graph of maximum palatal length plotted against fetal age

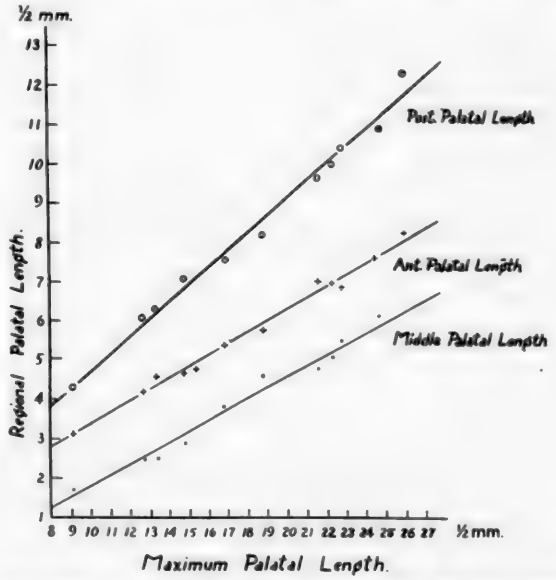

Fig. 7. Rate of growth in anterior, middle, and posterior divisions of palate in relation to maximum growth in length.

the early fetal months, the form of the palate exhibits more and more a tendency for divergent growth; the palates assumes mesuranic, brachyuranic and hyperbrachyuranic forms, such as one encounters frequently in the adult. Pantographic tracings of the morphological

Fig. 8. Palate of 9 month fetus showing surface markings and their relationship to crypt walls. H, anterior sulcus; G, antero-lateral sulcus; F, lateral sulcus; CC, dental groove; DD, lingual groove; 1, crypt for medial incisor; 2, crypt for lateral incisor; 3, crypt for canine; 4, crypt for first deciduous molar; 5, crypt for second deciduous molar; 6, posterior limiting membrane of alveolar arch.

sequence of palate types (*Fig. 5*) not only supports this view but illustrates the relative growth attained during the fetal periods. Table I presents the distribution of palate types in the various age groups. Of 27 dolichouranic palates seen, 22 were distributed between ages 3 to 6 fetal months. In the 5th fetal month the percentage of dolichouranic palates is still more than half the total number of cases

of this type examined, and from this period on the percentage rapidly decreases. The number of mesuranic palates found shows wide variation; 1 case appears in the 4th fetal month while the largest group appears between 6 and 7 fetal months. In the brachyuranic type, 1 case appeared in the 5th month while 12 cases were found

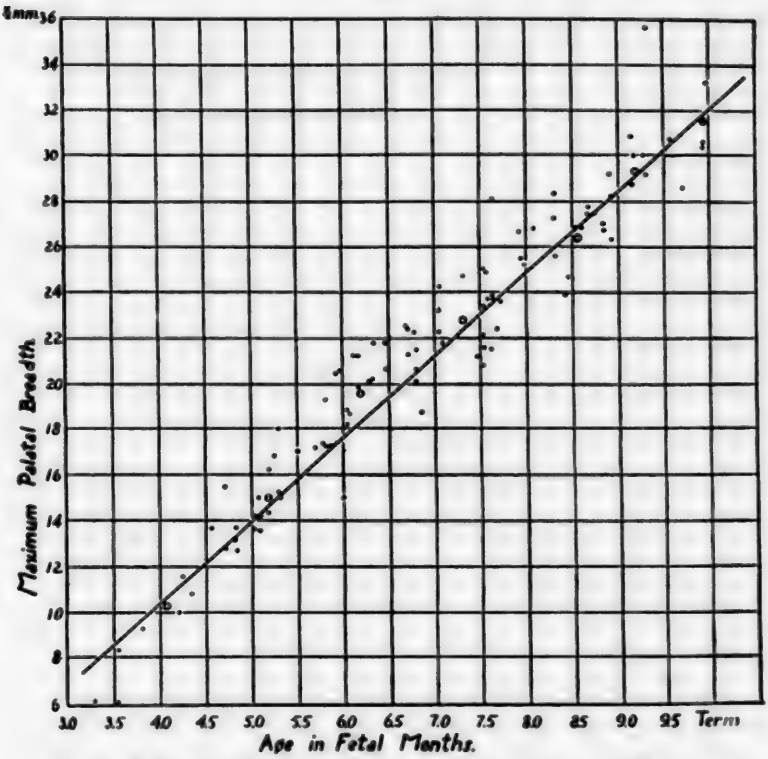

Fig. 9. Graph of maximum palatal breadth plotted against fetal age

between 7 and 8 fetal months. Only 10 cases of hyperbrachyuranic palates were seen and these occurred during the latter half of the fetal growth period. In the newborn and in postnatal life, the brachyuranic type of palate predominates.

The most characteristic feature of palate development is the appearance in early fetal life of an extremely large number of

dolichouranic palates. This fact is the basis of the hypothesis of progressive stages in the growth of the palate. It may be emphasized further that in the early fetal stages no divergent forms of palate types were found. It may be assumed, therefore, that the fetal palate is established as a dolichouranic type from which di-

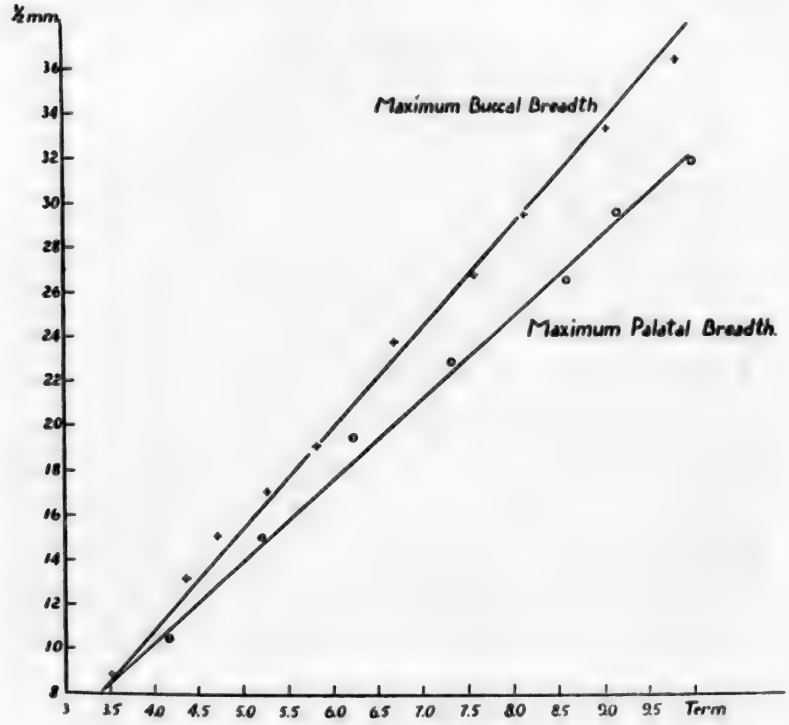

Fig. 10. Relation of rates of growth in length and breadth of palate during fetal period.

vergent forms develop. The palate form in the child or adult is elaborated from the dolichouranic type; *i.e.*, an adult brachyuranic palate developed from the primary dolichouranic type by passing through the mesuranic stage. An adult dolichouranic palate began as such in the early fetal development, remained undifferentiated throughout the growth period and persisted in the adult. A hyperbrachy-

uranic palate seen in the late fetal period represents a process of organized growth from the early fetal dolichouranic form. It must be admitted, however, that this hypothesis could be established only by direct observation on the same subject at intervals during development, an impractical approach in fetal studies.

It is interesting further to correlate palate types with the anterior arch curvature, as determined from the anterior palatal index values. It is evident from Table II that variability in the anterior arch curvature is general in each of the palate groups.

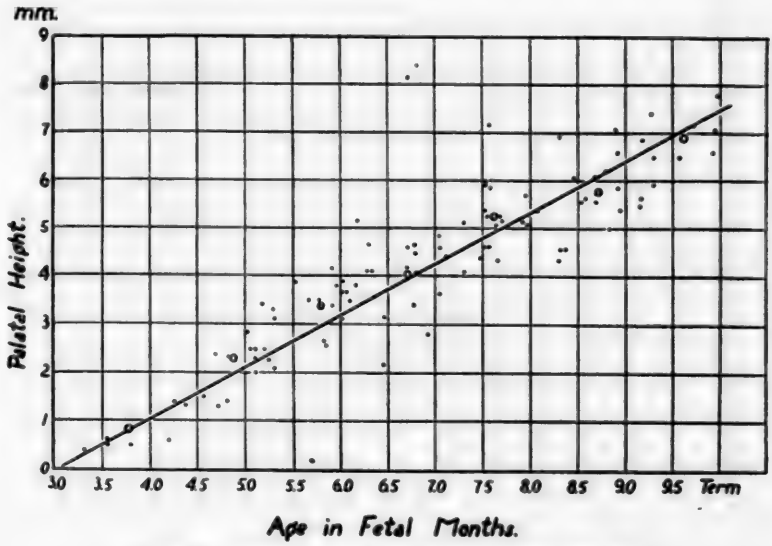

Fig. 11. Graph of palatal height plotted against fetal age

b. Growth in length of the palate. The relationship of length of the palate, plotted against fetal age, approximates a straight line (*Fig. 6*). This is indicative of a relationship to the constant growth of other structures during the fetal period. In *Fig. 7* the anterior, middle, and posterior palatal lengths are plotted against total length. The result as before exhibits linear growth. The anterior and middle palatal lengths are characterized by a parallel increase in growth, indicating a tendency for the anterior portion of the palate to increase in length at a uniform growth rate. More rapid growth occurs in

the posterior part of the palate; and the steeper slope of this curve indicates that the main trend of growth in length of the palate is in a posterior direction. This rapid growth of the palate posteriorly is associated with the migration backward of the fibrous membranes, limiting the alveolar arch posteriorly (*Figs. 8, 6*), and later becoming

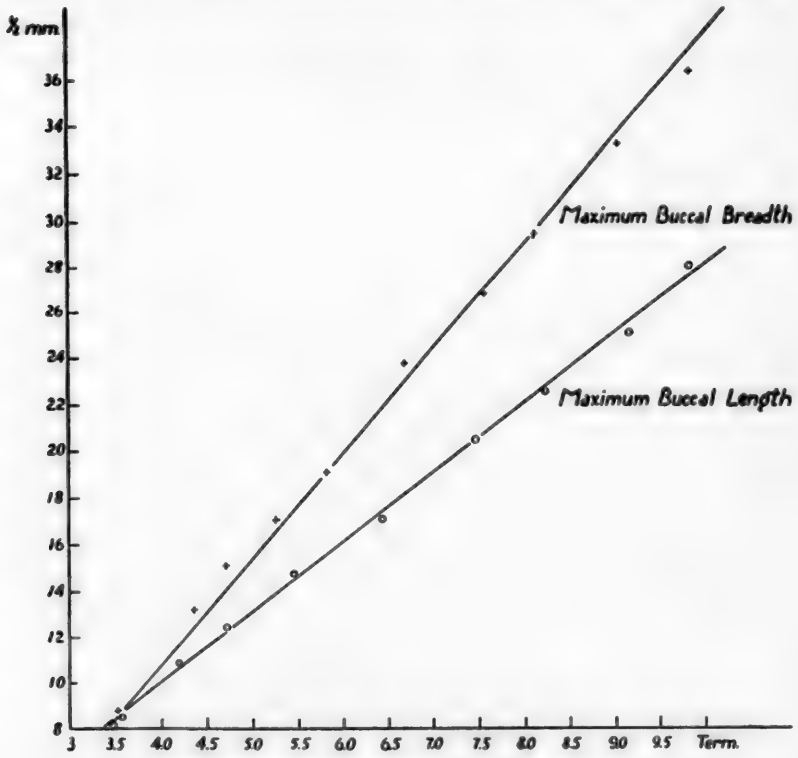

Fig. 12. Growth in length and breadth of the maxilla.

ossified to form the maxillary tuberosities. This more rapid growth of the palate posteriorly allows for an increase in the posterior alveolar arch to accommodate the permanent teeth.

c. Growth in breadth of the palate. In *Fig. 9* palatal breadth is plotted against fetal age. Here again a linear increase in growth is

associated with development of the palate. How can this linear increase in breadth be interpreted to account for the distinct variations of palate types observed during the fetal period? The form of the palate is governed to a large extent by alterations in breadth. It has been seen that in later fetal life variations of palate form are observed in fetuses of the same age. The relationship between length

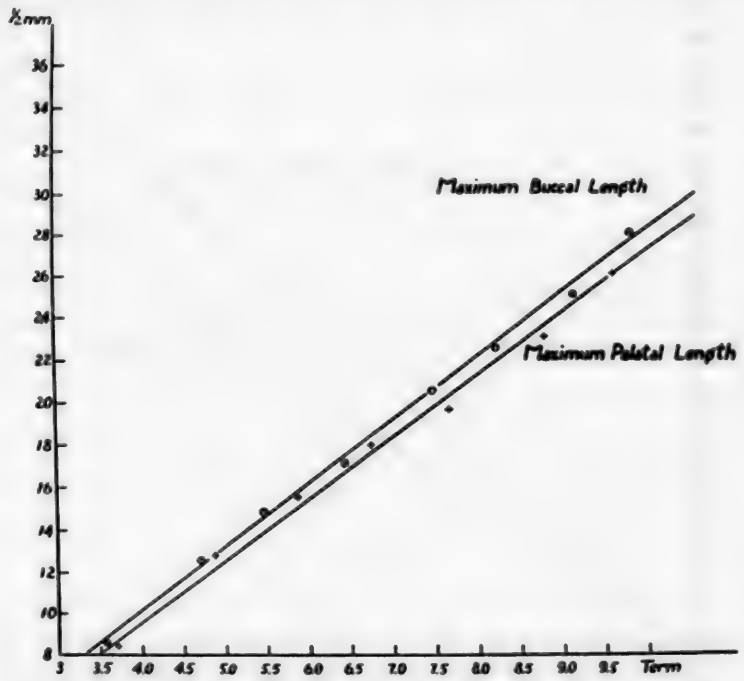

Fig. 13. Relation of growth rates in length of palate and maxilla

and breadth in the growth of the palate, given in *Fig. 10*, indicates that increase in breadth occurs more rapidly than increase in length. It is obvious that this more rapid growth in breadth contributes to the alteration of the palate form which is so marked in later fetal development and in postnatal life.

d. Growth in height of the palate. Associated with an increase in length and breadth of the palate is observed a corresponding increase

in height (*Fig. 11*). This growth in height, like the dimensional growths previously considered, is also linear. After embryonic development, when the lateral palatine processes have already fused to form the palate, a somewhat flat, unarched roof is associated with the dolichouranic forms seen in early fetal life. A fetus of 3.3 months

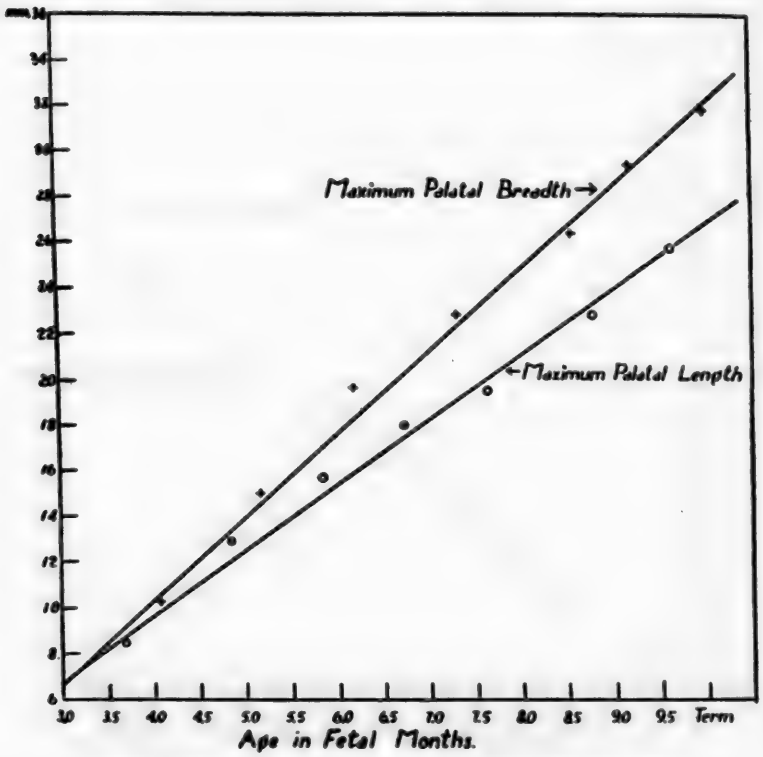

Fig. 14. Relation of rates of growth in breadth of palate and maxilla

showed a palatal height of .4 mm. The height of the palate increases steadily throughout the fetal growth period, so that in a fetus of 9.9 fetal months, the palatal height was 7.7 mm. Usually the maximum height occurred in the mid-line of the palate, but in many cases it was found to be displaced to one side. The increase in height of the palate which converts it into the arched form of fetal and postnatal

life is related to differences in the other dimensional growths. Several investigators have stated that this increase in height is probably concerned also with growth of the superior maxilla and increase in size of the vomer.

e. Growth in length and breadth of the maxilla. On plotting length and breadth of the maxilla against fetal age, the measurements are grouped lineally, indicating constant dimensional increases associated with growth in this region (*Fig. 12*). The characteristic feature of buccal or maxillary growth is a greater increase laterally than anteroposteriorly, dependent largely on the rate of growth of palate and alveolar arch.

f. Relation of palate growth to growth of the maxilla. The relation of palatal to maxillary growth is given in *Figs. 13* and *14*. In *Fig. 13* the relationship of growth in length of maxilla and palate is seen to be close. The rate of growth in length of maxilla and palate seem to adapt themselves simultaneously to changes in size and shape, and parallel linear growth curves are observed. *Fig. 14* indicates that the maxilla grows in breadth at a more rapid rate than the palate. This difference appears to be directly influenced by the growth of the alveolar arch and its adaptation to the developing teeth. The important fact thus appears that development of palate and maxilla is associated not so much with uniform growth in length, as with differences between its parts in growth in breadth.

SUMMARY

The development of the palate in the fetus is related to a series of growth processes. The most characteristic feature of palate growth in early fetal life, is a preponderance of dolichouranic palates. The fetal palate is established as a dolichouranic type, from which, by a series of organized linear growth changes, the palate types seen in postnatal life are developed: the mesuranic, brachyuranic and hyperbrachyuranic forms. Within each palate type general variability is observed in the anterior arch curvature.

Growth in length of the palate proceeds steadily throughout fetal life, but more rapidly in the posterior than in the anterior and middle portions, allowing for increase in the posterior alveolar arch to accommodate the permanent teeth. Increase in breadth is linear throughout fetal life, and proceeds more rapidly than increase in length.

This more rapid growth in breadth contributes to the alteration of palate form that appears prominently in later fetal and in postnatal life. Increase in height of the palate is also linear. A rather flat, unarched roof is observed in the early fetal period. The increase in height of the palate which converts it into the arched form is related to differences in the dimensional growths. Linear increase in length and breadth of the maxilla also occurs during fetal life, and, as in the palate, the increase is more rapid in breadth than in length. This is dependent largely on the rate of growth of palate and alveolar arch. The palate and maxilla exhibit parallel increases in rate of growth in length, but the maxilla grows more rapidly in breadth than the palate, perhaps directly influenced by adaptation of the alveolar arch to the developing teeth.

The writer wishes to thank Dr. M. F. Ashley-Montagu, of the College of Dentistry, New York University, and Dr. G. J. Noback, for making available the facilities of the Anatomy Department; Dr. W. M. Shanklin and Dr. H. Y. Rihan, of the American University of Beirut, Syria, for advice on preparation of this paper; and the Mortuary Division of the Department of Hospitals, New York City, for permission to secure the extensive collection of human fetuses which made this study possible.

REFERENCES TO LITERATURE

(1) Alkan, L. *Arch. f. Laryng. u. Rhin.* 10: 441, 1900.
(2) Ashley-Montagu, M. F. The form and dimensions of the newborn palate, unpublished manuscript, 1932.
(3) Eustachius, B. Libellus de dentibus, Venice, 1563.
(4) Franke, G. *Ztchr. f. Laryng. u. Rhin.* 10: 187, 1922.
(5) Hunter, J. The natural history of the human teeth, pp. 101, 102, 1803.
(6) Katz, J. Ueber die Gaumenform beim Neugeborenen, Unpublished Inaugural Dissertation in the Library of the Senchenbergische Institut Anatomie, Universitat Frankfurt a.M. pp. 1–23, 1923.
(7) Oudet, J. E. Footnote to J. E. Oudet's translation of Bell's edition of Hunter, John: Natural history of the human teeth, pp. 69–72, 1839.
(8) Scammon, C. E., and Calkins, L. A. The development and growth of the external dimensions of the human body in the fetal period, Minneapolis, Minn., p. 276 1929.
(9) West, C. The development of the gums and their relationship to the deciduous teeth in the human fetus. Contrib. to Embry., no. 79, 16: 39–46, 1925.

GOLD DECORATED TEETH FROM THE PHILIPPINE ISLANDS[1]

LESLIE F. RITTERSHOFER, D.D.S., M.S.

Ann Arbor, Michigan

While making a series of measurements to determine changes in the face and jaws on skeletal material in the Philippine collections of the Museum of Anthropology at the University of Michigan, it was learned that this collection contained a group of 43 human anterior teeth with small holes on the labial surface into which gold discs or plugs were inserted for decorative purposes. These examples of an unusual method of adornment were obtained by the 1922–1925 archaeological reconnaissance expedition sent by the University, under Dr. Carl E. Guthe, to the southern half of the Philippine archipelago. They were originally associated with burials located in three widely separated parts of the Visayan, or central, group of islands, namely the islands of Negroes, Masbate, and Samar. Regarding conditions associated with these finds, Dr. Guthe states,

"The great majority of the teeth bearing gold decorations was encountered while screening the deposits in two burial caves on small islands just off the west coast of Samar. The conditions and associations in both, which were in limestone formations, were essentially the same. The floors, although usually dry, had clearly been subjected to water action, probably during storms. The confusion resulting from natural disturbances was increased by visits of beasts and men. The bones of the skeletons had been scattered. The caves had apparently been used a long time as places of burial, for the collections, obtained largely by screening the deposits, included, in addition to the ornamented teeth and other skeletal remains, the following materials: fragments of stoneware and earthenware plates and bowls; sherds of greenish-brown glazed jars and of nativeware vessels; a few iron implements and handles made from antler fragments; and articles of adornment, including bracelets, rings, and beads of shell,

[1] Presented at the Thirteenth General Meeting of the International Association for Dental Research, Chicago, March 16–17, 1935; *J. Den. Res.* 15: 158, 1935.

stone, and gold. It may be noted in passing that some of the associated Chinese trade vessels found with the teeth appear to have been made during the Sung dynasty (960–1279 A. D.). Among the hundreds of disassociated human teeth were many that were discolored, probably largely by betelnut stain. Others showed evidence of having been filed, either to a point, or to flatten their labial surface."

Documentary evidence shows that the Filipinos decorated their teeth not many centuries ago. Antonio Pegafetta, Italian chronicler of Magellan's voyage, records having seen a native chief with gold spots on every tooth.[1] Senilla, in 1564–5, mentions a custom of decorating teeth with gold.[2] Pedro Chirino's "Relacion," published in Rome in 1604, describes the Filipinos' custom of staining and decorating their teeth with gold discs.[3] Morga, in 1609, also mentions this custom.[4] Father Diego de Bobadilla, in an unsigned and undated manuscript probably written in the years 1638–1640, wrote that the Filipinos cover the teeth with a black and glossy or flame colored polish; and thus their teeth become black, or as red as vermillion. In the upper row they make a little opening, and fill it with gold, which shows off to advantage on the black or red background of that polish.[5]

The writings of Juan Francisco de San Antonio, published in Manila between 1738 and 1744, seem to indicate that the Filipinos in general abandoned the custom before the middle of the eighteenth century.[6] The records of Dr. Louis Ottofy of Chicago indicate that a modified form of this peculiar type of adornment still persisted occasionally as late as the twentieth century.[7] Yet the custom of mutilating teeth for purposes of adornment seems to have disappeared in the Philippines before any known careful record was made of the process by which disfigurement was accomplished. Fortunately, travellers of recent years in other islands of Malaysia have described a still existing identical custom among the inhabitants of Borneo and Sumatra, with

[1] Blair and Robertson 33, 123.
[2] *Ibid.* 2, 223.
[3] *Ibid.* 12, 187.
[4] *Ibid.* 16, 78.
[5] *Ibid.* 29, 287.
[6] *Ibid.* 40, 327.
[7] Personal communication to Dr. Guthe.

the single difference that brass was used instead of gold.[8,9,10,11,12,13,14] Furness describes such a custom among the Ibans of Borneo.[11]

The custom of mutilating teeth for purposes of adornment is not confined to Malaysia. A variety of forms of tooth filing occur in many parts of the world. In New Guinea the natives file the teeth to points.[15] In central Africa filing to other forms is practiced.[16] At least two forms of tooth filing existed in Mexico, Central America, and various parts of South America.[17] But the custom of inlaying teeth, as described in this paper, is by no means so wide in its distribution. The only parts of the world outside of Malaysia in which it occurs are southern Mexico, northern Central America, and northwestern South America (Ecuador).[18] In these regions, the drilled holes were often of larger diameter than those here described, and in addition to gold the Indians used polished stones and ores as material for the plugs.

The large University of Michigan collection of teeth from the Philippines bearing gold discs or holes for their insertion warrants a detailed technical examination of the specimens in order to determine: (1) methods used in carrying out the operative procedures preliminary to actual insertion of the discs; (2) exact method of attachment; (3) character of the metal used; (4) shape in which the metal was available; (5) reason for the stains; (6) effects which such mutilation may have had upon the teeth themselves, the underlying pulps, and the surrounding soft tissues and bone; and (7) the effect of diet and the presence or absence of any methods of oral hygiene practiced.

Fig. 1 is a photograph of each tooth from the labial aspect. *Fig.* 2 is an x-ray from the mesio-distal aspect. The teeth are arranged in the same order in both figures, starting with no. 1 at the

[8] J. B. Davis, 289. Skull no. 279.
[9] H. Ling Roth 2, 78.
[10] E. H. Gomes, 38.
[11] W. H. Furness, p. 157.
[12] Alfred Maas 1, 150.
[13] *Ibid.* 373.
[14] von Nehring, p. 217.
[15] Bene van Rippen, 4.
[16] Bene van Rippen, 2.
[17] M. H. Saville; Bene van Rippen, 1 and 3.
[18] *Ibid.*

upper left. A few examples among these of conditions bearing on the questions raised will be discussed. Tables I and II summarize the conditions and characters observed on each tooth. In order to avoid undue repetition, it may be said that with but one or two exceptions all the teeth are stained a light brown, presumably from the chewing of betel nuts. The anterior teeth, for the most part, have strong marginal ridges on the lingual surface, creating a modified shovel effect. Concretions, mostly of the salivary type, are present in almost every case. More or less severe abrasion of the occlusal surface of the teeth is nearly always present.

Nos. 3, 6, 18, 24, and 39 illustrate the various designs found. These vary from a single hole to five, six, or seven, sometimes arranged in a triangular pattern. Nos. 4 and 11 illustrate the two types of plate patterns found. One is cut in the shape of a four leaf clover; the other is simply an irregular thin plate of gold.

Figs. 1 and 2 illustrate a number of conditions found frequently. For example, the first tooth on the upper left (No. 1), an upper central incisor, shows the distribution of stain characteristic of many of these teeth; the labial surface is quite free from stain for perhaps 3 mm. from the cemento-enamel junction. This condition would indicate that a marked marginal gingivitis[19] had existed previous to staining of the teeth. In this tooth only one of the six holes contains a metal disc, which in this case is semi-circular. The x-ray of this tooth (No. 1, *fig.* 2) indicates that the base of the disc barely rests in the dentine and that the pulp apparently had undergone considerable recession.[20]

No. 2 is a canine with extremely heavy deposits which extend apically below the cemento-enamel junction, so that considerable recession of the gums must have occurred. Both x-ray and photograph show the extreme abrasion of the occlusal surface.

No. 3, an incisor, illustrates a condition consistently present, namely, the hole is round and has a round base. In this particular case there is nearly a pulp exposure (*fig.* 2). The distribution of the stain indicates that a marked marginal gingivitis existed.

No. 4 is an upper left canine, the entire labial surface covered by a thin sheet of gold measuring but 0.15 mm. in thickness. Upon removal this sheet or plate showed no evidence of cementation; but the under surface was found to be in the form of a short gold tube, broken open at the pulpal end, and apparently formed by punching a small gold rod into the plate directly over the hole. The split open end of the tube indicates this method of attachment. The labial surface was smoothed off and highly polished.

No. 8 is an incisor illustrating a common condition: the primitive operator drilled through enamel and dentine into the pulp. *Fig.* 2 indicates this condition very clearly. The slight flange on the labial side of the disc shows that it had been hammered into the hole. This tooth is a striking illustration of decorative *filing*. Both mesial and distal corners are filed, giving the crown a V-shaped appearance. Nos. 16, 23, and 42 show crowns also filed V-shaped.

---

[19] Kronfeld, R. 291, 305.

[20] Kronfeld, R. 35.

No. 9 is a lateral incisor with a variation in form commonly known today as a peg lateral. The contact points are excessively worn by long hard usage in mastication.

No. 11 is an incisor covered by one of the four-leaf patterns of gold plate. The plate in this case is much thicker (0.33 mm.) than the one on no. 4, and was secured by the same method, except that in this case the hole was carried completely through the pulp chamber and ends in the opposite wall (*fig.* 2), probably not a painless operation. The great size of the pulp chamber indicates early death of the tissue.[21]

No. 15 shows another example of probable gingivitis, the stain being high off the cemento-enamel junction. In this case the incisal edge is so severely abraded that two of the decorative holes have been opened from the occlusal.

No. 16, an incisor with the crown filed V-shaped, has a single large hole which, though indistinctly shown in *fig.* 2, penetrated the pulp chamber. The root apex shows considerable hypercementosis. A marked hypercementosis may also be observed on the apices of nos. 23, 29, and 36.

No. 18 is an incisor illustrating apical absorption and hypercementosis. It has three labial holes which are shallow and hold no gold. The pulp chamber had been opened from the lingual surface by a large hole. One may speculate as to whether the hole might have served to drain an abscess. The conditions indicate the presence of infection and the location of the hole contraindicates any decorative purpose.[22]

Nos. 5, 8, 11, 16, and 23 in *fig.* 2 are teeth in which the pulp chamber was penetrated by the decorative hole. In no. 23, the large size of the pulp and the small, slightly worn contact points, indicate that the tooth was yet young when lost by infection.[23]

No. 24 is a canine showing a very deeply filed labial surface holding seven irregular round gold discs which rest just within the dentine. This illustrates ideal conditions for retention

No. 36 is an incisor with absorption of the root apex, hypercementosis, and a large pulp, conditions indicating infection and early loss of the tooth.

No. 37 is a canine holding a plate attached as previously described. The only new condition here is that the plate has been so deeply polished that the outlines of the pins are exposed. The location of the contact points in this tooth is such that some irregularity of tooth arrangement is indicated. It should be noted in *fig.* 2 that severe abrasion of the incisal edge is the rule.

Spectroscopic analysis[24] of these discs demonstrated the presence of gold, silver, and copper. One disc from no. 8 and two from no. 43 were used for this analysis. The color and a specific gravity of about 15 indicates a preponderance of gold.

Detailed examination of each specimen, indicates that the holes, although varying from 1 to 2.3 mm. in diameter, were drilled by a cylindrical instrument with a round end or base, and that it was purely by chance that the operator stopped short of the pulp in any instance. The great variety in shape and size of the discs is no doubt accounted for by the fact that they were used more or less as found in nature,

[21] Kronfeld, R. 37, 291.
[22] Kronfeld, R. 154.
[23] Kronfeld, R. 36.
[24] Analysis made by John Manley, Physics Department, University of Michigan.

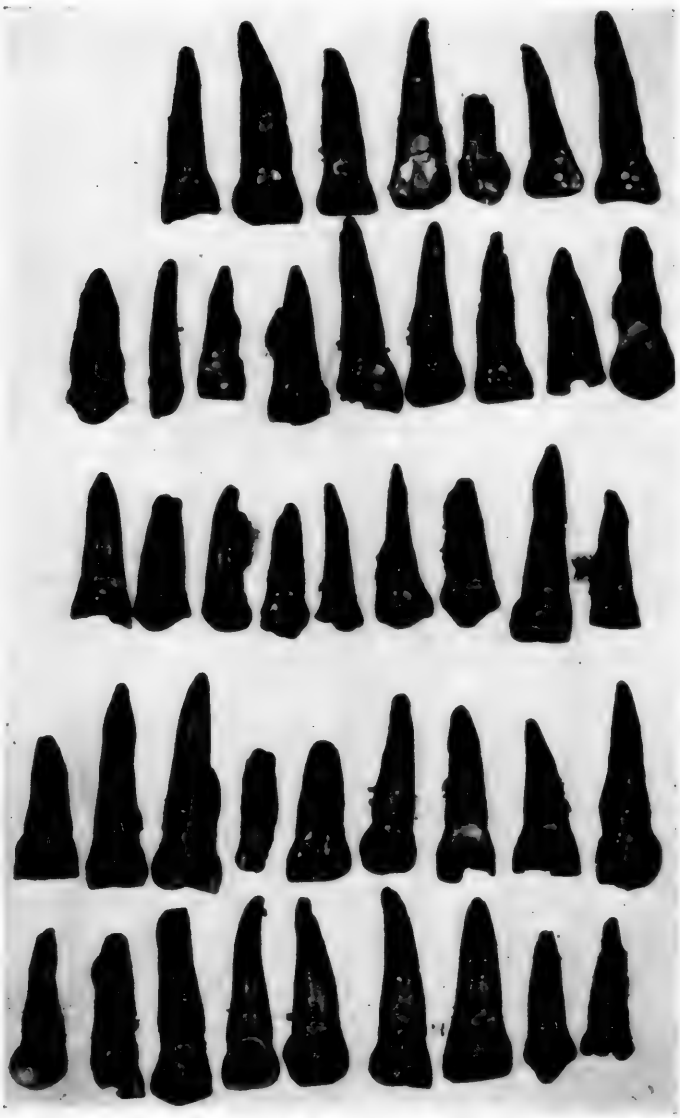

Fig. 1. Teeth numbered from left to right. Top row, 1–7; second row, 8–16; third row, 17–25; fourth row, 26–34; last row, 35–43.

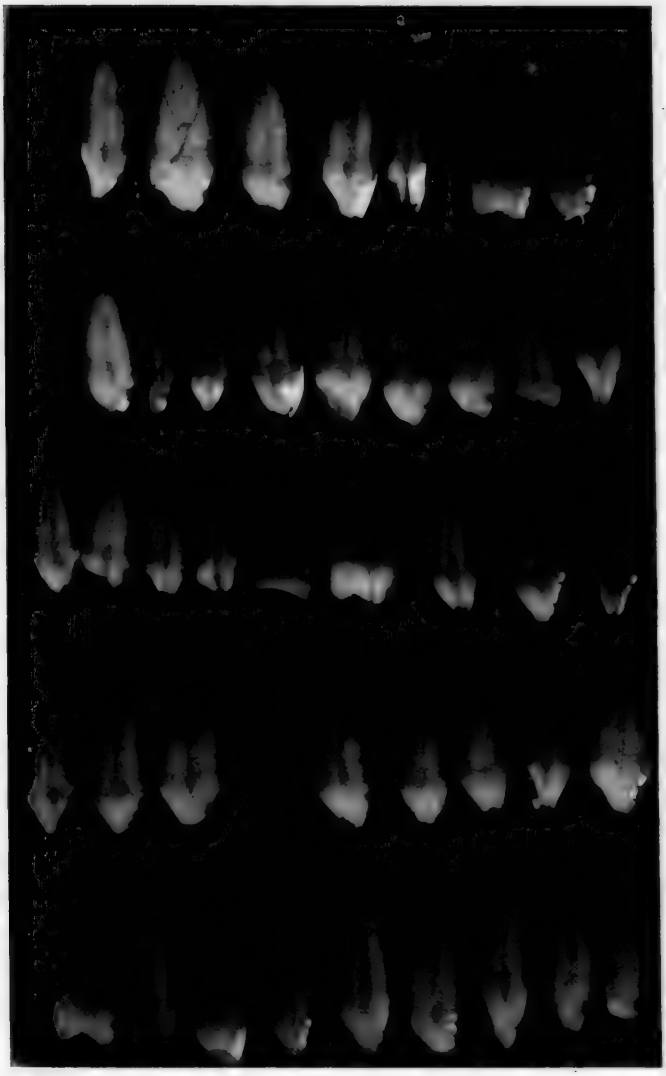

Fig. 2. Teeth numbered from left to right. Top row, 1–7; second row, 8–16; third row, 17–25; fourth row, 26–34; last row, 35–43.

TABLE I

Data on teeth and decorations

TOOTH NO.	CATALOG NO.	TOOTH NAME	DIAMETERS IN MILLIMETERS				PLATE THICKNESS IN MM.
			Tooth crown		Hole	Gold disc average	
			Mesio-distal	Labio-lingual			
1	B 1-123	L. C. Incisor	7.60	6.69	1.0	1.0	
2	B 1-123	R. Canine	8.84	9.11	1.5	1.0	
3	C 47-10	R. C. Incisor	7.50	7.48	2.0		
4	C 47-9	L. Canine	7.40	7.23	1.8		0.15
5	C 47-9	R. L. Incisor	5.30	5.15	1.5	1.5	
6	C 7-71	R. 1st. Prem.	7.37	8.64	1.0		
7	C 7-71	R. Canine	7.92	7.70	1.0	1.5	
8	C 7-71	L. C. Incisor	7.88	7.11	1.2	2.0	
9	C 7-71	L. L. Incisor	4.29	4.40	1.2	2.0	
10	C 7-71	R. L. Incisor	5.86	5.83		1.0	
11	C 7-71	L. C. Incisor	8.50	7.25	1.6	1.0	0.33
12	C 7-72	R. C. Incisor	8.18	8.23	1.2		
13	C 7-72	L. Canine	7.64	7.80	1.0		
14	C 7-72	L. Canine	7.15	7.83	1.7		
15	C 7-72	R. C. Incisor	7.43	7.31	2.2		
16	C 7-72	R. C. Incisor	8.56	7.00	1.8		
17	C 7-72	R. C. Incisor	7.84	6.78	0.8		
18	C 7-72	R. C. Incisor	7.22	6.98	2.0		
19	C 7-72	L. L. Incisor	6.20	6.58	0.9		
20	C 7-72	L. L. Incisor	6.05	5.85	1.6		
21	C 7-72	R. 1st. Prem.	6.18	8.33	2.2		
22	C 7-72	L. 1st. Prem.	7.20	9.30	1.9		
23	C 7-74	L. C. Incisor	7.33	6.83	1.5		
24	G 9-15	R. Canine	7.65	7.68	1.2	1.2	
25	G 9-15	R. L. Incisor	6.25	6.39	1.2	1.4	
26	G 9-15	R. C. Incisor	7.93	6.71	0.8		
27	G 9-15	L. Canine	7.71	7.97	0.8		
28	C 2-19	R. Canine	8.80	9.10	2.3		
29	C 2-19	R. L. Incisor	6.70	6.20	2.3		
30	C 2-19	L. C. Incisor	8.12	7.98	2.0		
31	C 2-19	R. Canine	7.40	7.18	1.2		
32	C 2-19	R. C. Incisor	7.40	7.03	1.0		
33	C 2-19	R. C. Incisor	8.60	7.15	1.0	1.0	
34	C 2-19	L. Canine	8.42	9.10	1.5	1.5	
35	C 2-19	R. 1st. Prem.	7.11	9.00	1.7		
36	C 2-19	L. C. Incisor	7.40	7.00	1.8		
37	C 2-19	R. Canine	7.98	8.20	1.0	1.0	0.10
38	C 2-19	L. Canine	7.49	7.50	1.0		
39	C 2-19	R. Canine	8.04	8.00	1.4	1.4	
40	C 2-19	R. Canine	7.96	8.12	1.4	1.4	
41	C 2-19	L. C. Incisor	8.75	8.21	2.2		
42	C 2-19	L. L. Incisor	7.65	6.50	2.0		
43	C 2-19	R. L. Incisor	6.70	6.43	1.0	1.0	

TABLE II

Data on Individual Teeth

Tooth Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Caries																																										
Calculus on crown	x	x	x		x	x		x	x	x		x	x			x	x	x	x		x	x	x	x			x	x															
Calculus on root also				x			x				x	x																			x			x			x						
Stained lightly	x	x						x	x			x	x	x	x		x		x	x	x	x		x	x	x	x			x			x	x	x		x			x		
Stained heavily			x	x	x	x	x			x						x		x					x																				
Stained 2–3 mm. below cemento-enamel jct.				x	x	x	x		x		x	x				x			x		x	x		x	x	x	x		x	x		x		x			x		x	x		
Stained 1–2 mm. below cemento-enamel jct.	x														x		x	x		x			x																			x	x
Contact points worn little																	x									x		x					x		x								x
Contact points worn severely	x	x	x			x	x	x		x	x	x	x	x	x	x			x		x	x																					
Abrasion ending in enamel					x	x		x	x	x												x	x									x										
Abrasion ending in dentine	x	x	x	x			x				x	x	x	x		x			x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Abrasion ending in pulp															x			x																									
Pronounced marginal ridges on lingual	x	x	x	x	x	x	x	x		x		x	x	x	x	x		x	x	x			x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x
Apical absorption																		x		x																							
Hyper cementosis																x							x					x															
Labial surface filed flat	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		x	x	x	x				x		x	x			x	x				x	x
Labial surface filed concave		x																x									x	x	x														
Labial filing ending in enamel	x		x	x		x			x	x	x	x	x	x	x	x			x	x	x	x	x				x	x		x	x	x		x		x					x	x
Labial filing ending in dentine		x					x	x	x								x							x	x	x			x				x										
Number of holes in labial surface	6	5	1	1	1	4	6	4	3	5	1	1	1	1	1	1	7	3	1	1	1	1	1	7	7	6	6	1	1	2	1	1	7	5	1	1	3	6	5	6	1	1	1
Number of holes ending in enamel											1							1				1					6			1	1												
Number of holes ending in dentine	6	5	1	1	1	4	6	3	3	4			1	1			5	3	1		1			7	7	5				1		1	7	5	1		3	6	5	6			1
Number of holes ending in pulp								1		1	1				1	1	2			1			1			1		1	1							1				1	1		
Reduced pulp chamber	x		x	x			x	x	x	x	x	x		x	x	x	x		x		x	x		x	x					x	x	x	x	x		x	x	x	x	x	x	x
Holes having round base		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Number of gold discs retained	1	1			1		4	1	2	2	1													7	7							1	2			3	6	5	6			2
Shape of disc round	x	x	x	x	x		x	x	x	x	x													x	x								x			x						
Shape of disc irregular	x						x	x		x	x													x	x																	x
Crowns filed "V" shape								x								x						x																				

and were not subjected to any preparation beyond the possible selection of one of approximate size. There is every reason to believe that the Filipinos obtained the gold used in this manner by placer mining in some of the Philippine streams, from which gold may be obtained today. Judging from the flanges on the labial sides of the disc, and the absence of anything to indicate an auxiliary attachment other than mechanical, one may assume that the discs were simply hammered into the holes. Retention in many cases was made doubly easy because the hole was larger at the base than at the surface. The locations of concretions point to gingival recession[25] in at least 20 per cent of the teeth. The distribution of stains indicates marginal gingivitis in at least 70 per cent. Many of the pulps were exposed during the operation of drilling holes. Furthermore, there is evidence that suppurative conditions existed in a number of instances. The extreme wear of incisal edges and contact points implies excessive movement, probably associated with chewing of betel nut and of harsh and gritty food. The presence of salivary deposits on all surfaces of the teeth suggests that this chewing was the total extent of any oral hygiene practiced. One tooth (no. 37) illustrates malocclusion of the anterior teeth. No. 9 shows the presence of a common variation in form. Nos. 2, 8, and 22 indicate the presence of pyorrhetic conditions accompanied, in no. 2, by severe alveolar absorption. Despite the fact that not a single instance of caries was found in this collection, other pathological conditions present indicate that these people lost their decorated teeth early.

The evidence presented demonstrates that these inlaid decorative discs were attached by a partial combination of the modern gold foil filling and cast inlay methods. There is nothing to indicate that the discs and plates were for any other purpose than decoration, inasmuch as no disc occurs where it could not be seen; and, because of the total absence of caries, no need for restorative measures could have existed.

REFERENCES TO LITERATURE

(1) Blair, Emma Helen and Robertson, J. A. The Philippine Islands; 1493–1898, 55 vols. Cleveland, 1903–1909.

(2) Davis, J. B. Thesaurus Craniorum; Catalogue of the Skulls of the various Races of Man, in the collection of J. B. Davis. London, 1867.

[25] Kronfeld, R. 303.

(3) Furness, W. H., 3rd The Home-life of Borneo Headhunters. Philadelphia, 1902.
(4) Gomes, E. H. Seventeen Years among the Sea Dyaks of Borneo. London, 1911.
(6) Kronfeld, R. Histopathology of the Teeth and their Surrounding Structures, Philadelphia, 1933.
(7) Maas, Alfred Durch Central-Sumatra. 2 vols.
(8) Nehring, H. von *Zeit. Ethnol.* 14: 1882.
(9) Rippen, Bene van 1. *Den. Cosmos* 59: 861, 1917.
——— 2. *J. Allied Den. Soc.* 13: 1, 1918.
——— 3. *J. Allied Den. Soc.* 13: 219, 1918.
——— 4. *J. Allied Den. Soc.* 13: 365, 1918.
(10) Roth, H. L. The Natives of Sarawak and British North Borneo. 2 vols. New York, 1896.
(11) Saville, M. H. *Amer. Anthrop.* 15: 377, 1913.

ROUND CELL SARCOMA OF THE MANDIBLE[1]

PAUL C. KITCHIN, M.S., D.D.S., AND CHARLES A. DOAN, M.D.
Dental Research Laboratory, and Department of Medical Research, Ohio State University

As has been stated by Cahn, (2) "when the patient comes to the dentist with a loose tooth, or several loose teeth, the condition is immediately attributed to peridontoclasia. In the vast majority of cases the diagnosis is correct, in a few exceptional cases the cause is more serious." Fortunately, the possibility of the loosening of teeth being due to sarcoma is not great but that possibility should always be kept in mind by the dentist when such cases present. Galt, (3) states that in the Surgical Clinic of Vienna only thirty-one cases of sarcoma of the jaws were collected over a period of twenty years, and of one hundred malignant tumors of the jaws, Ochsner found only sixteen to be sarcomas.

Before any operative interference, x-ray studies should always be made of these loose teeth cases. Careful examination of the films will, in the case of sarcoma, give a distinctly different picture than that found in peridontoclasia and root abscesses. Bloodgood (4) said in this regard, "The roentgenogram of the ordinary root abscess is so distinct that little risk is run by extracting the tooth, but if the films reveals a lesion about the tooth that differs in any respect from the ordinary root abscess, one should not extract that tooth unless he is prepared to make a frozen section of the material that can be scraped from the root." The same author (5) stated, ".... preservation of the life of the patient may depend on the first treatment when the central tumor of the jaw is exposed and it is my opinion that preoperative irradiation is another measure that will act as a safeguard."

In discussing the differentiation between root abscesses and malignant growths in the jaws, Geschicter (6) says, "Such radicular ab-

[1] Presented at the 14th General Meeting of the International Association for Dental Research, Louisville, Ky., March 14–15, 1936, *J. Den. Res.* 15: 338, 1936.

scesses must be carefully distinguished in the roentgenogram from more irregular areas of destruction about the roots of the teeth. Too often such a loose tooth is extracted and the tooth, together with the tissue clinging to it, discarded without any further investigation. In some cases, too many in fact, a growth protrudes from the tooth socket after an interval of a few weeks and is either carcinoma or sarcoma. The records in the Surgical Pathological Laboratory of the Johns Hopkins Hospital show that in most cases of either squamous cell carcinoma or sarcoma in such regions, the initial chance of diagnosis depended upon either the proper x-ray interpretation of the area of resorption or upon a biopsy of the tissues clinging to the root of the extracted loose tooth, and that both opportunities for an early and correct diagnosis were missed."

Sarcoma is a disease both of youth and of old age, hence a localized loosening of the teeth in a young person should immediately arouse suspicion of a malignancy. The prognosis, according to Lyons (7) is very unfavorable, because by the time the patient consults a dentist the malignant growth has probably already metastasised by way of the blood and lymph streams to other areas. Nevertheless recognition of the condition by the dentist is imperative in order that all that may be accomplished by deep x-ray or radium therapy, can be realized by having such treatments started at the earliest possible moment.

The case described was in a male of 18 years. The chief complaint, at the time of admission, was swelling and tenderness of the left mandible. A month previous to this time the patient had what he considered to be a gum boil in the area of the lower left second bicuspid. The condition became progressively worse with a marked loosening of the second bicuspid. His local dentist removed the tooth. The lump on the jaw persisted and the first molar became loosened and was subsequently removed by the same dentist. In both sockets an excessive growth of new tissue developed. X-rays were then taken and the patient referred to an oral surgeon, who made further roentgenographic study. Recognizing an unusual condition the patient was sent for consultation. Thus we first saw this case two weeks following the extractions of the original loose teeth and about four weeks after the patient first became aware of the local swelling. The appearance of the sockets at this time is shown in *Fig. 1.*

The patient's past history was one of normal health, except for the usual childhood diseases; smallpox occurred at 4 years. One year previous he had been kicked in the stomach and had suffered fracture of some ribs and the clavicle with no apparent residual pathology. His weight was 160 pounds. The family

history was negative. The available oral roentgenograms which had been taken when the sockets failed to heal promptly showed conditions in various areas of the jaw bone, particularly around the recent extractions and in the upper right bicuspid and molar areas, which were quite different from those usually observed in association with loose teeth. The complete loss of the lamina dura around the second upper right bicuspid and the unusual appearance of the supporting bone suggested neither destruction due to root abscesses nor to peridontoclasia. *Fig. 2* is an enlarged reproduction of the roentgenogram of this area. A similar reproduction of the film from the lower left bicuspid and molar region, where the original extractions were made, is shown in *Fig. 3*. Here there is the beginning of an attack on the peridental membrane and roots of the second molar, the destruction of the lamina dura in the lower third of the first molar socket, the complete loss of the same in the bicuspid socket and an unusual condition of the supporting bone in both socket areas. The mandibular canal is much enlarged. One of the most outstanding features is the lack of any evidence of caries or peridontoclasia in the second molar which could account for the tissue changes around the roots. The root tips do not suggest root abscess. In this case they are sharpened rather than rounded, indicating a rapidly progressive lateral resorption. This is the picture of the action of a highly malignant growth, apparently arising from the mandibular canal and involving an otherwise sound tooth and its supporting structures.

The growth from the sockets was a roughened, greyish pink, granulation-like tissue. There was a marked swelling over the region of the mental foramen on the left side.

Laboratory studies gave negative Wassermann and Kahn reactions; urine analysis, specific gravity of 1021, acid reaction, albumen and sugar negative, and some bacteria present; blood Ca—9.8 mg., P—4.1 mg. per 100 cc. of serum; icteric index—8; cell volume—37; erythrocyte fragility range—.412–.300 and the sedimentation rate—51 mm. in two hours.

The early blood picture indicated a moderate degree of secondary anemia as shown by the lowered erythrocytes and hemoglobin. Eosinophiles were 6 per cent and lymphocytes were low. The blood picture was compatible with the toxic influence of a malignant growth.

A biopsy from the growth in the lower left second bicuspid socket showed "on supravital examination a very cellular tumor growth. The predominating cell type was a medium sized mononuclear cell with vesicular nucleus, containing one to three nucleoli and with cytoplasm free of mitochondria and neutral red bodies. No giant cell types were seen. An occasional phagocytic clasmatocyte was present. A very occasional neutrophilic granulocyte was found. The impression gained from this survey and confirmed in the studies of the fixed sectioned material is that we are dealing with a round cell sarcoma, the primitive character of the cells suggesting a radio-sensitive growth." *Fig. 4* is an area from the fixed sectioned biopsy material showing the character of the sarcoma cells and the frequency of mitoses.

The X-ray Department reported on the case as "multiple sarcomatous lesions

in the mandible." The treatment suggested was cross firing with massive doses of deep-x-ray. Radium therapy was contraindicated because of the diffuse character of the lesions, and radon seed implantation was financially impossible.

Surgical consultation confirmed the medical opinion against radical surgery.

A representative of the Eye, Ear, Nose and Throat Department described a considerable enlargement of the left tonsil with no pain but with an impairment of speech. The tonsil was smooth, hard and pushed out into the throat by a hard indurated peritonsillar growth. The tumor growth was deep red with some engourgement of the superficial vessels.

Immediate deep x-ray therapy was instituted. A massive irradiation (550 "R") was directed from the left at the site of the socket growths and the tonsil. This was followed within twenty-four hours by a noticeable resorption of the new growth in both locations. The lump over the mental foramen disappeared. The effect of irradiation on the tooth socket growths is shown in *Fig. 5*. After treatment the patient was dismissed with instructions to report back in two weeks.

He returned six days later with swelling in the lower right and upper left bicuspid areas. These enlargements were pale in color and firm to pressure. The upper right molars were loose, allowing lateral movement with a crackling feeling. The lower left socket areas showed no swelling or growth. Deep x-ray therapy (500 "R") was given to the right side of the face and was followed shortly by a recession of the swellings. The patient was again dismissed with instructions to return if the symptoms reappeared.

He was next seen sixteen days later. An upper-left and a lower-right bicuspid which were so loose that they were a source of annoyance to the patient were removed under nitrous oxide-oxygen anaesthesia. Considerable tissue remained attached to the tooth roots. One was fixed, sectioned longitudinally and stained (*Fig. 6*). The advance of the tumor growth, invading the peridental membrane and destroying the alveolar and supporting bone is plainly shown. On the day following these two extractions, deep x-ray therapy (550 "R") was administered to the left side of the face and the patient again discharged to his home.

Eighteen days later he reported back with marked recurrence of buccal and lingual swelling and with the upper right maxillary area, from the cuspid back, containing loosened teeth. The right mastoid region was tender. The right side of the mandible was swollen and there was a growth, of one week standing, from the lower right bicuspid socket. The upper left bicuspid socket showed no tumor growth and the original lower left sockets were quiescent. Deep x-ray therapy (550 "R") was given to the right side of the face with a complete reduction of swelling and pain within 24 hours. The patient was kept under observation for the next ten days in the hospital and there was no recurrence of symptoms during that period. He was then allowed to return home.

It was 24 days before he was seen again and by this time the tumor had recurred in all four extraction areas, with much swelling in both the lower and upper right jaws and some swelling in the upper left bicuspid area. All the teeth in the swollen areas were loosened and there was also swelling in the right temporal region. The patient had suffered a progressive anemia with increasing

dyspnea and weakness since the last treatment. General physical examination disclosed a tumor growth in the right groin. Biopsies were taken from the growth in the lower right bicuspid socket and from the metastatic node in the groin. The pathologist's report on both tissues was round cell sarcoma. At this time the temperature and pulse were both well above normal. Another application of deep x-ray therapy (300 "R") was made on the right side of the face and the patient hospitalized. Increasing pain necessitated the use of opiates. General metastases were now becoming apparent. Eighteen days later bleeding was noticed from the stomach, skin nodules began to appear on the face and body, especially on the abdomen, and edema of the extremities developed. Anemia became more marked. Death occurred four months after the first tooth extractions. No post mortem examination was permitted.

The hematologic study in this case presents several interesting features. The lymphocytes were reduced in absolute numbers with a relative and absolute neutrophilia. The monocytes remained for the most part within normal limits. The hemoglobin and erythrocyte count were already markedly reduced when the patient first came under our observation. There was marked anisocytosis, poikilocytosis and achromia of the red cells with generalized microcytosis, i.e., a typical hypochromic, microcytic anemia.

Despite intensive iron therapy (iron and ammonium citrate, 2 grams t.i.d.) there was a continuous progressive fall in hemoglobin and red cells with reciprocal reticulocytosis during the succeeding three months. The picture was one primarily of toxic inhibition of hemopoiesis. Some suggestion of metastatic displacement was reflected in the recovery of red cells, hemoglobin and granulocytes following irradiation therapy on March 30. The extremely rapid destruction of these radio-sensitive tumor elements, as observed directly in the new growths in the oral cavity, make the sudden release of increased bone marrow elements attributable to a similar phenomenon. The tendency to early and extensive metastasis renders successful therapy in this type of cellular tumor impossible with present facilities.

Aside from the factual information and photographic material on round cell sarcoma of the mandible presented here, we believe that there has been shown in the treatment of this case the cordial and complete coöperation of dental and medical facilities which is so necessary in those cases where both professions are equally involved.

REFERENCES TO LITERATURE

1. Cahn, L. R. *Den. Cosmos* 77: 484, 1935.
2. Idem. *Ibid.* 75: 203, 1934.
3. Galt, C. B. *J. A. D. A.* 21: 1442, 1934.
4. Bloodgood, J. C. *Ibid.* 20: 1790, 1933.
5. Idem. *Ibid.* 20: 1996, 1933.
6. Geschickter, C. F. *Den. Cosmos* 75: 529, 1933.
7. Lyons, C. J. *J. A. D. A.* 20: 3, 1933.

Fig. 1. Growths in sockets of lower left mandible two weeks after extraction of "loose" teeth and four weeks after patient first became aware of condition.

Fig. 2. Enlarged reproduction of oral x-ray showing complete loss of lamina dura around upper second right bicuspid and partial loss of same in first bicuspid and first molar. Note the unusual appearance of supporting bone. These teeth were loose.

Fig. 3. Enlarged reproduction of oral x-ray of lower left bicuspid and molar areas show beginning of attack on apices of second molar and surrounding alveolar bone and peridental membrane.

Fig. 4. Photomicrograph of biopsy material taken from growth in lower left bicuspid socket. Note character of sarcoma cells and frequency of cell division.

Fig. 5. Showing reducing effect of x-ray therapy on growths in sockets of lower left mandible. They soon reappeared however.

Fig. 6. Photomicrograph of longitudinal section through extracted upper left bicuspid showing advance of sarcoma down peridental membrane with accompanying destruction of membrane, lamina dura and supporting bone.

Fig. 1

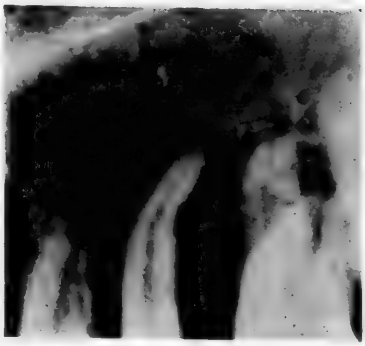

Fig. 2

Fig. 3

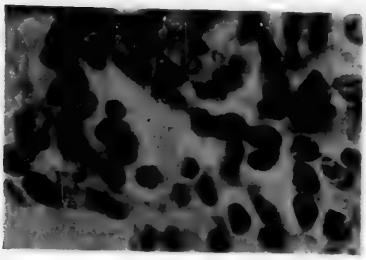

Fig. 4

Fig. 5

Fig. 6

THE CAPE-KITCHIN MODIFICATION OF THE CELLOIDIN DECALCIFYING METHOD FOR DENTAL ENAMEL

CHARLES F. BÖDECKER, D.D.S.; F.A.C.D.

Laboratory of Oral Histology, School of Dental and Oral Surgery, Columbia University, New York, N. Y.

The demonstration of the organic or protein matrix of human dental enamel has been greatly simplified by the method suggested by Cape and Kitchin (1). Their contribution has added further evidence of the non-crystalline nature of the enamel residue isolated by decalcification and makes the demonstration of some parts of the protein matrix of the enamel easy. This simple method will probably aid many in attaining a better comprehension of the structure of the enamel matrix and its progressive changes during dental caries.

The presence or absence of the organic or protein enamel matrix is of far-reaching importance in directing the investigation of the problem of dental caries; this is brought out by the following question: Is dental enamel a uniform, impermeable, wholly mineral structure (as claimed by some investigators) or, does it contain protein matter allowing a certain degree of permeability? If enamel is wholly mineral, then the destruction of the teeth appears to be the result of a comparatively simple mechanism, i.e. the action of an oral acid. In this case, the activity of dental caries can be explained readily on the supposition of variations in oral environment (food retention, bacteria and saliva). On the other hand, if there is evidence that dental enamel contains a protein matrix which allows a certain degree of permeability, the mechanism of dental caries may be more complex. The possibility exists that the enamel can be affected by the pulp from within as well as by the saliva penetrating the surface of the tooth. Evidence is accumulating that such permeability exists in the teeth of children and young adults.

The original method of decalcifying dental enamel and thus defining the organic matrix was described at the beginning of this

century (2a, 2b). Evidence for the presence of this matrix, composed of enamel rod sheaths, tufts and lamellae was accepted abroad shortly thereafter (3). Structures which previously had been regarded as cracks in the enamel were defined by means of this method as sheets of organic matter and were named "enamel lamellae" by the author (2a, 2b, 2c). Malleson (4) developed a like method of decalcifying enamel in 1924 and showed similar organic structures. Certain modifications have been made in the technique of this method.

The belief that human dental enamel is devoid of a protein matrix persisted for some time in our country and is, in fact, still considered to be true by some authorities in spite of the recent work of Chase (5). The first indication of a change of opinion took place in 1923 (6). At this time a representative group of men, charter members of the International Association for Dental Research, signed the following statement:

"At the meeting of the New York Section of the International Association for Dental Research held in New York City on the 7th day of December 1923, the matrix of the enamel was demonstrated by Dr. Charles F. Bödecker. We, the undersigned, hereby certify that we have examined this decalcified matrix of human adult enamel under the microscope. We have teased it with needles and find that it is soft, elastic, sponge-like and reticular in nature. Physically it does not show the characteristics of mineral matter, no trace of brittleness or hardness being evident in any of the specimens of enamel matrix presented to us tonight. What its true nature may be is a matter for the chemist to determine."

Signed by: "William J. Gies, Geo. Wood Clapp, V. H. Jackson, Milo Hellman, Bissell B. Palmer, Jr., Arthur H. Merritt, M. L. Rhein, Theodr. Blum, F. C. Kemple, J. Lowe Young, A. Berger, P. R. Stillman, Herman E. S. Chayès, Andrew J. McDonagh, L. M. Waugh."

Doubt was expressed by a few members of the group that the isolated structures might be some product of the acid celloidin employed in decalcifying the specimens; this doubt was allayed shortly thereafter by the chemical analyses of Gies (7) who defined it as a protein.

A description of the Cape-Kitchin method of decalcification is contained in the following extract from their paper (1):

"Parts of thin tooth sections varying from 20 to 30 micra were placed on a two and one-half by two inch glass slide and a heavy line of white petroleum jelly was traced on the slide in such a way that it would act as a complete seal for the periphery of a 24 x 50 mm. #1 cover glass. Into the container enough acid celloidin, prepared according to Bödecker's directions, was poured and the cover glass placed on the rectangle of petroleum jelly and pressed down onto the section."

The preparation of the acid celloidin is now simple. In the original method, Schering's celloidin was used, which when in solution, does not mix well with the water present in nitric acid. In preparing acid celloidin with the Schering product, the entire mass solidifies so that it is necessary to re-dissolve it. Now, DuPont's Parlodion is used in place of Schering's celloidin.

To prepare acid celloidin[1] DuPont's Parlodion is cut into small cubes and dissolved in acetone-free methyl alcohol to make a thick, syrupy solution. To 200 cc. of this solution add, under constant stirring, a mixture of:

Methyl alcohol (acetone-free)	90 cc.
Nitric acid. Sp. G. 1.42	9 cc.

The strength of the acid in the celloidin may be varied; a one to three percent solution has been found best for the Cape-Kitchin method of decalcifying the average tooth section.

The principal reason why dental enamel was erroneously regarded as a wholly mineral structure was its reaction to ordinary decalcification. If enamel is placed in a five percent aqueous solution of nitric acid, it apparently disappears. The reason for this is that the protein matrix is in an exceedingly finely divided state. The slightest movement of the watery fluid in the dish tears the matrix from the dentin and it sinks to the bottom of the vessel as impalpable fragments. The advantage of the celloidin method is that the enamel is decalcified while embedded in the syrupy celloidin, thus preventing its distortion to a certain degree.

The Cape-Kitchin method has definite advantages over the original method, inasmuch as it always makes possible the demonstration of the protein enamel matrix in relation to the tooth as a whole. Thus the enamel lamellae and tufts can be seen attached to the surface of the dentin. Another advantage of this method is that the technique is so simple, results being obtained on the same day, that its use will become common. A disadvantage of the Cape-Kitchin modification is the thickness of the finished specimens; this precludes the use of

[1] The designation "celloidin" is retained even though the solution is made with "parlodion," because the former is the name by which the standard embedding technique is known.

the immersion lens and makes photomicrographs of the organic structures somewhat indistinct (*Fig. 1.*) The specimens can not be preserved permanently. By the use of the original method, exceedingly thin sections (3 micra) of the enamel matrix can be made, owing to the double embedding of celloidin-paraffin. These photograph well, even under the high enlargement (*Fig. 3*). But usually little or no dentin can be included (as this tissue does not section well when embedded in paraffin) and only isolated pieces of enamel can be treated successfully by the celloidin decalcifying method. The above discussion shows that both methods have their uses and that the Cape-Kitchin technique will do much to make the demonstration of the enamel matrix simple.

The presence of a considerable amount of matrix in specimens of some teeth led a few investigators to believe that the enamel residue, seen under the microscope, is only partially decalcified. The use of polarized light, as advised by Cape and Kitchin and corroborated by Applebaum settles this point nicely. This procedure differentiates the crystalline portion of the enamel from that entirely devoid of mineral salts.

The method of defining the progress of decalcification of enamel is as follows: the tooth section is immersed in acid celloidin as advised by Cape and Kitchin and observed between crossed Nicols with a 24 mm. objective on the stage of a polarizing microscope. At first, the structure of the enamel is seen clearly, because the crystalline elements are double refracting (birefringent), but the picture changes soon as the nitric acid attacks the specimen. When the crystalline elements of the enamel have been dissolved completely, this tissue is no longer double refracting, i.e. the region that has been decalcified is no longer visible (*Fig. 1*, DEM). With normal light however, the organic matrix is seen to be composed of the organic rod sheaths (*Fig. 2*, DEM). Photomicrographs of sections decalcified by the Cape-Kitchin method are not very clear because the residual matrix can not be stained. Staining would require the removal of the celloidin and this procedure results in the complete derangement and ultimate loss of the matrix. It is comprehensible naturally, that the entire section, including the decalcified protein matrix, is bathed in a *solution* of the

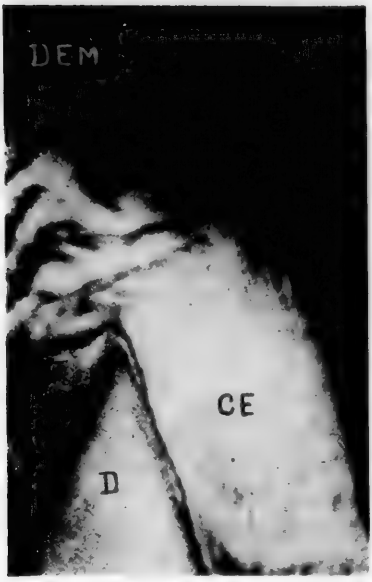

Fig. 1

Fig. 2

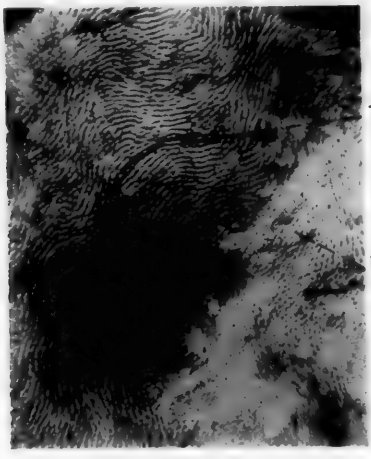

Fig. 3

Fig. 1. Longitudinal, ground section of human lower bicusped, age 70, decalcified partially by Cape-Kitchin decalcifying method and photographed with polarizing equipment under crossed Nicols. Only that part of enamel containing mineral salts is visible on account of its birefringence (CE). The decalcified enamel matrix (DEM) is invisible. D = dentin. (Original magnification 65 ×)

Fig. 2. Same tooth section photographed with white transmitted light at the same stage of decalcification as in *fig. 1.* Decalcified enamel matrix (DEM) is clearly visible showing enamel rod sheaths. (Original magnification 65 ×)

Fig. 3. Enamel completely decalcified with celloidin decalcifying method showing protein matrix. Details of enamel rod sheaths with occasional Tomes processes (TP) located centrally in the rods and tufts (T) composed of thickened rod sheaths. (Original magnification 400 ×)

mineral salts, abstracted from the enamel and dentin during the decalcification process. It is, therefore, in one sense, not "decalcified." These salts, being in solution, are not visible, even with normal light. It seems evident therefore, that the delicate residual structures present after decalcification, are not mineral, in addition other tests have shown them to be organic or protein in character.

The observations of Chase (5) on the varying amounts of protein matrix isolated by decalcification of different specimens of enamel, offer an explanation for the divergent figures found in the chemical analyses of this structure by different workers. The old investigations of von Bibra (8) show 3 to 5 percent of organic matter whereas the recent and more exact technique employed by Rosebury (9) places the amount of matrix in his specimens at 0.30 to 0.54 percent. Chase shows that the acid resisting residue of enamel (organic or protein matrix), varies with the age and degree of developement of the teeth. Consequently it is evident that chemical analyses will give either high or low amounts of enamel matrix dependent on the selection of the type, age and condition of the specimens.

SUMMARY

The Cape-Kitchin modification of the celloidin decalcifying method has the advantage of simplicity. By this means a greater number of investigators will become convinced of the presence of the grosser organic or protein matrix in human dental enamel.

The presence or absence of the protein matrix of the enamel affects the entire concept of dental caries for:

a) its ABSENCE would make the enamel almost completely impermeable to saliva and prevent fluids from penetrating this structure from the pulp by way of the dentin. On the other hand:

b) its PRESENCE would explain the permeability of the enamel, thus allowing the saliva, as well as the dental lymph to exert during youth, a beneficial action on the teeth.

REFERENCES TO THE LITERATURE

(1) Cape, A. T., and Kitchin, P. C. *J. Am. Den. Ass. 17;* 193, 1930.
(2a) Bödecker, C. F. *Zeitsch. f. wissen. Mikroscopie, 22;* 190, 1905.
(2b) *Ibid. Den. Review, 20;* 3, 1906.

(2c) *Ibid. J. Am. Den. Ass. 10;* 1164, 1923.
(3) FLEISCHMANN, L. *Zeitschr. f. wissen. Mikroscopie, 25;* Heft 3, 1908.
(4) MALLESON, H. C. *Brit. Den. Journal, 45;* 601, 1924.
(5) CHASE, S. W. *J. Am. Den. Ass. 22;* 1343, 1935.
(6) BÖDECKER, C. F. *J. Den. Res. 6;* 117, 1924–26.
(7) BÖDECKER, C. F., AND GIES, W. J. *Proc. of Soc. for Experimental Biology & Medicine, 22;* Dec., 1924.
(8) VON BIBRA, E. Chemische untersuchungen ueber die knochen und zaehne des menschen, Schweinfurt, Kunstverlag, p. 262, 1844.
(9) ROSEBURY, T. *J. Den. Res. 10;* 187, 1930.

SALIVARY CHOLESTEROL[1]

FRANCES KRASNOW, A.M., PH.D., AND EDITH B. OBLATT, A.B.

Guggenheim Dental Clinic, New York City

Cholesterol has been considered an active participant in the mechanism of various pathological conditions. Increased amounts in blood have been reported for kidney disorders, arteriosclerosis, hypertension, obesity, diabetes, and obstructive jaundice. Acute infections such as typhoid fever, scarlet fever, pneumonia, erysipelas, and syphilis frequently give rise to a marked decrease in blood cholesterol—the higher the fever, the lower the cholesterol content. Cholesterol analysis applied to other body fluids remains practically unexplored. A few investigators have studied spinal fluid (3, 4, 5, 6), but there seems to be no previous record of any attempt to ascertain its presence in saliva. The present paper gives a method for the quantitative determination of cholesterol in saliva, analyses showing the distribution of values generally as well as that obtaining for dental caries and erosion.

Method of Analysis. Preliminary tests proved that the technique of Myers and Wardell, with little modification, could be employed for the estimation of even the minute amounts of cholesterol in saliva (1, 2). As finally adopted, 4.0 cc. of fresh saliva in a 75 cc. casserole, is evaporated over a water bath to approximately 1 cc. and treated as described in the procedure for blood cholesterol (1). After extraction, the flask containing the extract is removed from the hot plate when the level of $CHCl_3$ in the extraction chamber is one-half the height of the siphon tube. The extract is evaporated to about 5 cc., filtered through fat-free Whatman #50 filter paper to a 10 cc. graduate, capped with filter paper and allowed to evaporate spontaneously at room temperature to 1.0 cc. To this is added 0.02 cc. of H_2SO_4 (sp. gr. 1.84) and 0.4 cc. of acetic anhydride,

[1] Preliminary report of some of the data was published in the *J. Den. Res.* 14: 226, 1934. Cholesterol in dental caries and erosion was presented as part of Clinical and Biochemical Studies of Dental Erosion and Abrasion by Isadore Hirschfeld, Frances Krasnow and Edith B. Oblatt at the meeting of the New York Section of the International Association for Dental Research, June 1936.

the mixture shaken, and placed in the dark for ten minutes. Micro-cups and plungers are used, and the color developed matched against naphthol green B which has been standardized against cholesterol. Because a brown coloration frequently develops when the extract is mixed with H_2SO_4 and acetic anhydride, a red disc is employed for matching. Such discs frequently necessitate correction factors, which always obtained in our earlier work. Recently, however, an adjustment was arranged which obviates the necessity of any corrections.

Results. Duplicate determinations checked within limits to be expected for the concentrations existing in saliva, as follows (values are mgm. for 100 cc.): case I-21, 2.6, 2.8; case H.T., 5.7, 6.1; case O-7, 9.0, 9.0. The analyses thus far carried out[2] are detailed in Table I.

There is a fairly wide range of variation—the lowest content being 2.3 mgm. per 100 cc. and the highest 50.0 mgm. Clinically normal individuals (identified in Table I by "I") who have had no dental disease, have salivary cholesterol contents falling between 2.3 and 9.4 mgm., per cent. Very few cases have yet been relegated to this group and so the general average 5.4 mgm. per cent (or 7.9 mgm. for postabsorptive samples) may be used as the normal figure only tentatively.

Table I includes also determinations obtained in a comparative series of salivas from subjects suffering with active caries and erosion (abrasion). The figures deduced from the analytical data are summarized in Table II.

In tabulating the results, our attention was drawn to differences between cases with alkaline saliva and those with acid saliva. Treated as separate groups the two types gave averages which placed the alkaline set lower and nearer to the normal level, but raised and removed the acid group farther from that value. Thus witness 5.3 ± 2.1 and 9.2 ± 0.7 mgm. cholesterol per 100 cc. of alkaline saliva in contrast to 15.7 ± 2.9 and 13.5 ± 2.0 in acid saliva from erosion and caries patients, respectively. If not subdivided, the average of each type is above the normal by an amount which

[2] We acknowledge with appreciation the kindness of Dr. Isadore Rosen in offering the facilities of his laboratory at the New York Post Graduate School and Hospital and that of Dr. A. S. Rosen for aid in the analyses.

TABLE I

General distribution of salivary cholesterol values

CASE	SEX	AGE	DATE	COLLECTION				MG. PER 100 CC.	DISTRIBUTION OF VALUES[1]	
				Stimulation	Time	Period	cc.		Range	Per cent
I-28	M	22	1–15–32	Paraffin	5:20[4]	30	25	2.3		
39 E	F	17	10–15–35	Unstimulated	Postab[5]	30	25	2.5		
I-21	M	21	1–13–32	Paraffin	5:00	—	25	2.7		
S-15	M	21	1–12–32	Paraffin	5:00	—	25	2.9		
S-31	M	22	1–21–32	Paraffin	5:50	10	23	3.1		
S-11	M	21	1–11–32	Paraffin	5:30	15	20	3.2		
I-13	M	20	1–11–32	Paraffin	5:20	10	20	3.2		
34	F	30?	12–28–34	Unstimulated	Postab	40	28	3.3		
I-16	M	21	1–12–32	Paraffin	5:00	—	20	3.6		
S-30	M	19	1–11–32	Paraffin	5:00	—	25	3.6		
31 E	F	20?	12–18–34	Unstimulated	Postab	30	19	3.7		
40 E	F	33	10–15–35	Unstimulated	Postab	75	25	3.8		
S 26	M	21	1–22–32	Paraffin	5:00	—	25	3.9		
S 14	M	19	1–13–32	Paraffin	5:00	—	25	4.0		
I 25	M	20	1–14–32	Paraffin	5:00	15	25	4.0		
S 18	M	19	1–13–32	Paraffin	5:00	—	25	4.1		
32 E	F	47	12–18–34	Unstimulated	Postab	30	26	4.4		
11 I	F	10	12– 3–35	Unstimulated	Postab	45	45	5.1		
41 E	M	43	10–15–35	Unstimulated	Postab	50	25	5.6		
H T-I	F	5 mos.	12–15–33	Unstimulated	Postab	30+30[6]	4.5+3.5	6.1		
S-23	M	21	1–22–32	Paraffin	4:30	90	33	6.1		
19 I	F	5	12– 3–35	Unstimulated	Postab	130	36	6.2		
35 E	M	41	5–14–35	Unstimulated	Postab	60	24	6.3		
9 C	F	9	10–29–35	Unstimulated	Postab	45	50	6.5		
17 C	F	11	11–19–35	Unstimulated	Postab	60	46	6.5		
21 C	F	7	12–10–35	Unstimulated	Postab	140	45	6.5		
10 E	F	25	10–29–35	Unstimulated	Postab	30	50	6.6		
S 22	M	20	1–14–32	Paraffin	5:45	25	35	6.8		
I 24	M	20	1–14–32	Paraffin	5:15	30	25	7.0		
I 27	M	21	1–15–32	Paraffin	5:00	—	25	7.0		
S 29	M	23	1–15–32	Paraffin	6:10	5	25	7.1		
45 E	F	32	2–11–36	Unstimulated	Postab	150	45	7.2		
15 CE	F	24	11–19–35	Unstimulated	Postab	30	49	7.3		
46 E	M	34	2–11–36	Unstimulated	Postab	135	45	7.7		
48 I	F	20	2–11–36	Unstimulated	Postab	95	45	7.9		
30 C	F	21	1–21–36	Unstimulated	Postab	130	45	8.3		
47 C	F	30	2–11–36	Unstimulated	Postab	90	45	8.6	2.3– 8.6	52.9
O-7	F	35	7–19–34	Unstimulated	Postab	265	18	9.0		
26 CE	M	34	1–14–36	Unstimulated	Postab	65	45	9.1		
28 C	M	11	1–21–36	Unstimulated	Postab	105	45	9.2		
6 I	F	7	3–17–36	Unstimulated	Postab	115	62	9.4		
23 C	M	8	12–10–35	Unstimulated	Postab	60	45	9.6		
24 C	F	8	12–10–35	Unstimulated	Postab	50	35	10.0		
3 C	F	9	3–17–36	Unstimulated	Postab	75	65	10.0		
27 C	F	22	1–28–36	Unstimulated	Postab	78	47	10.3		
54 E	M	54	5–12–36	Unstimulated	Postab	115	60	10.7		
8	F	8	10–29–35	Unstimulated	Postab	105	50	10.9		
16 C	F	15	11–19–35	Unstimulated	Postab	55	48	10.9		
30 C	M	24	2–18–36	Unstimulated	Postab	130	50	10.9		
18 E	M	25	12– 3–35	Unstimulated	Postab	70	45	11.0		
51 E	F	16	5–12–36	Unstimulated	Postab	—	60	11.1		
22 C	F	7	12–10–35	Unstimulated	Postab	150	20	11.5		
37 E	F	12	6–25–35	Unstimulated	Postab	75	25	11.6		
44 E	F	35	7– 1–35	Unstimulated	Postab	155	25	11.6		
29 C	F	13	1–21–36	Unstimulated	Postab	135	45	12.6		

TABLE I—*Concluded*

CASE	SEX	AGE	DATE	COLLECTION				MG. PER 100 CC.	DISTRIBUTION OF VALUES[a]	
				Stimulation	Time	Period	cc.		Range	Per cent
53 C	F	20	3–17–36	Unstimulated	Postab	135	50	13.1	8.7–15.0	27.1
51 E	F	41	5–12–36	Unstimulated	Postab	130	40	15.3		
14 C	F	13	11–12–35	Unstimulated	Postab	125	30	15.4		
25 EC	F	42	1–14–36	Unstimulated	Postab	160	45	15.6		
42 E	F	26	6–25–35	Unstimulated	Postab	75	25	15.8		
57 E	F	47	6– 2–36	Unstimulated	Postab	90	60	15.9		
60 P	F	49	6– 2–36	Unstimulated	Postab	110	23	16.7		
43 E	F	26	6–25–35	Unstimulated	Postab	130	25	16.8		
13 C	M	17	11–12–35	Unstimulated	Postab	140	34	18.6		
59 E	M	14	6– 2–36	Unstimulated	Postab	105	29	19.4		
20 C	M	22	12–10–35	Unstimulated	Postab	140	45	19.6	15.1–21.4	14.3
49 E	F	21	2–11–36	Unstimulated	Postab	90	13	27.3	21.5–27.7	1.4
55 C	F	6	6–2–36	Unstimulated	Postab	110	25	33.0		
36 E	M	53	5–21–35	Unstimulated	Postab	120	24	33.3		
56 P	F	32	6– 2–36	Unstimulated	Postab	162	48	34.5	27.8–34.5	4.3
58 E	F	38	6– 2–36	Unstimulated	Postab	210	19	50.0		

[a] The difference between the lowest value 2.3 and the highest value (not including 50.0) was divided arbitrarily into five equal intervals varying from each other by 6.4.

[c] Evening collections were made approximately four hours after the noon meal.

[b] Saliva from physically normal subjects in postabsorptive state was collected in the morning after a good night's rest, before washing mouth, brushing teeth, smoking, or breakfast, a 1½ to 2-hour period having elapsed between rising and collection, this interval including a fifteen-minute rest at laboratory (40–50 cc. collected).

[d] To obtain enough saliva for this analysis, two collections of two consecutive mornings were mixed. That secured the first day was stored in the ice chest. Previous experimentation indicated no deterioration of the cholesterol when thus treated.

TABLE II

Comparative levels of salivary cholesterol for dental caries and erosion
Analyses on total unstimulated saliva

TYPE OF CASE	NUMBER OF CASES	TOTAL CHOLESTEROL AVERAGE, MGM. PER CENT	PERCENTAGE DISTRIBUTION OF VALUES WITH REFERENCE TO THE NORMAL AVERAGE RANGE		ODDS AGAINST RANDOM OCCURRENCE OF DEVIATIONS CALCULATIONS BASED ON D/P.E.[7]
			Below	Above	
Normal	6	7.9 ±1.0[8]			
Erosion	21	13.7 ±2.5	33	57	5.4
Alkaline saliva	4	5.3 ±2.1	75	25	1.0
Acid saliva	17	15.7 ±2.9	24	65	9.9
Caries	22	11.8 ±1.3	14	73	8.5
Alkaline saliva	9	9.2 ±0.7	22	67	1.2
Acid saliva	13	13.5 ±2.0	8	77	9.9

[7] D/P.E. as suggested by Pearl (7) is used to indicate the odds against random occurrence of a difference between two series. D = difference between the two classes under consideration, P.E. = probable error.

[8] This value is the standard deviation, $\sigma = \sqrt{\frac{\Sigma d^2}{N(N-1)}}$. Σ = sum, d = difference of an individual value from the mean, N = total number of analyses in any series.

does not give overlapping. When, instead of considering the averages, the percentage distribution below and above the average normal range is tabulated, a high percentage of cases falls beyond the high normal value and this percentage is increased for the subgroups with acid saliva. To test further whether these differences are significant, computations were made for the odds against their random occurrence. Values for caries-acid saliva and for erosion-acid saliva deduced by employing the formula, D/P.E. (7) approach significance. There is apparently a very close agreement of the three methods for the evaluation of analytical results differentiating the salivary composition accompanying abnormal dental conditions from the normal. It is interesting to note that simultaneous quantitative studies of other salivary constituents indicate general relationship of cholesterol to pH, protein and lipid phosphorus.

CONCLUSIONS

1. The method proposed for analysis of salivary cholesterol yields good reproducibility. Duplicate determinations check within 10 per cent.

2. The series of cases thus far studied present marked variation in salivary cholesterol—the limits being 2.3 to 50.0 mgm. per 100 cc.

3. There is apparently a distinct tendency to increased cholesterol in saliva (especially when acid) of caries and erosion susceptible individuals.

REFERENCES TO LITERATURE

(1) Krasnow, F., and Rosen, A. S. *J. Lab. Clin. Med*, 14: 967, 1929.
(2) Myers, V. C., and Wardell, E. L. *J. Biol. Chem.* 36: 147, 1918.
(3) Osnato, M., Killian, J. A., Garcia, T., and Mattice, M. R. *Brain* 50: 581, 1927.
(4) Rosen, I., and Krasnow, F. *Arch. Derm. Syph.* 13: 506, 1926; *Ibid.* 20: 75, 1929; Rosen, I., Krasnow, F., and Lyons, M. A., *Ibid.* 27: 383, 1933.
(5) Rosen, I., Krasnow, F., and Notkin, J. *Arch. Neur. Psych.* 28: 399, 1932; *Ibid.* 29: 155, 1933.
(6) Wells, H. G. Chemical Pathology, 1925. W. B. Saunders Company, Philadelphia.
(7) Pearl, R. Introduction to Medical Biometry and Statistics. W. B. Saunders Company, Philadelphia, 1930.

MICROLOGY OF GINGIVAL INFLAMMATIONS[1]

THEO. B. BEUST, D.D.S., M.D., F.A.C.D.[2]

University of Louisville, Louisville, Ky.

Regardless of the character of the irritation, the conditions found in inflamed gingival pockets have much in common. Considerable masses of microbial deposits are almost invariably present and, excluding the existence of mechanical trauma, when the organisms are removed and prevented from returning the symptoms disappear. Little is known about these microbes and few practitioners are aware of the incredible number that inhabit the gingival pocket. If dentists could be sufficiently impressed with the importance of these organisms, the loss of teeth through periodontal disease could largely be prevented or postponed. Hartzell (1) writes, ".... the majority of the dentists of the country think that bacteria and their ferments are purely incidental to periodontoclasia; whereas they are fundamental to it, ...". Owing to confusion in terminology it is very difficult, if not impossible, to give an intelligible account of the microbiology of an oral inflammation without first discussing the history, nomenclature and morphology of the organisms concerned. This will be done briefly, using as an introduction the family *Spirillaceae*.

From the standpoint of frequency, the *Spirillum sputigenum* of Miller occupies a conspicuous position in inflammatory lesions of the mouth. Clark, 1879 and Rappin, 1881 (2) were probably the first to describe this form, although W. D. Miller's work made known generally its common occurrence in the mouth. The name *Comma bacillus*, because of its resemblance to the *Cholera vibrio*, is also applied to this organism. Differentiating names for the spirilla were introduced into bacteriologic science in 1838 when Ehrenberg (3)

[1] Read at the Fourth Annual Meeting of the Dental Section of the American Association for the Advancement of Science, St. Louis, January 4, 1936.

[2] Work supported, in part, by a grant from the Research Commission of the American Dental Association.

applied to the rigid spirals the term spirillum and to the flexible forms the name spirochete. Thirty-four years later (1872), F. Cohn used this part of Ehrenberg's classification and added the term vibrio, to distinguish the shorter spirals. Migula, whose 1895 classification is largely used in recent literature, characterized the spirilli as rigid and motile with polar flagella while the flexible spirochete was said to be motile by means of unknown appendages. These appendages have since been described although the views concerning them vary greatly.

This history, coupled with the universal use of these names during more than forty years makes recent attempts to substitute such names as *Borrelia* or *Treponema* for the flexible mouth spirals deplorable. Enough confusion has reigned heretofore. Miller gave us a fairly good description of the *Spirillum sputigenum*, but left much to be conjectured when he dealt with the spirochetes. Under the term *Spirochete dentium* (*denticola*), he includes two forms. These have since been separated by Hoffmann and von Prowazek (4), the larger being named *Sp. buccalis*, the smaller *Sp. dentium*. Miller, moreover, used the names spirochete and spirillum interchangeably, a mistake that is still being followed. Now we are confronted with the terms *Spironema*, *Treponema*, *Borrelia* and *Vincent's spirochete* as applied to the mouth spirochetes. If someone will publish the precise points of differentiation between these and the spirochetes long known as inhabitants of the mouth, matters will be simplified. I believe that we should not depart from the older nomenclature until additions to our knowledge of the morphology of these organisms justify such a course (5).

Before considering the pathogenicity of these organisms, mention should be made of the fusiformed bacilli. These are almost universally associated with the mouth spirilli in chronically inflamed gingival crevices. The spirillum, spirochete and the fusiformed bacilli form an almost inseparable trinity. In numerical occurrence these organisms vary with the smear and with the case; here one, there the other predominating.

It has been common practice to approach the problems created by these organisms by the cultural method. In the case of the organisms now being considered, this method has not yet been

attended with success. W. D. Miller, who had the advantage of an association with Robert Koch, worked industriously at their cultivation in the early '80's. Inoculations of such material under the most variable conditions (1905–1908) have been made by the author without success. It is true that their successful culture has been reported, but the author has not found a case where the experimental results have been verified by careful comparison with those grown in the mouth. If we were to assume that in rare cases a limited artificial cultivation has succeeded, the fact remains that they will not grow on the ordinary media. If, for example, an artificial substratum is inoculated from secretions taken from a pupal abscess or gingival crevice, colonies of streptococci commonly appear. Etiologic significance is forthwith attached to the finding. Careful smear examinations made from tissues that have given streptococcus growths frequently show also other organisms, such as comma forms, spirochetes and fusiform organisms. Animal experiments, undertaken with colonies of cocci from such sources, have given rise to theories of grave import to the dental profession. In lesions that are in open communication with the oral cavity, such as gingival pockets, the non-cultivatable organisms so completely overshadow the cocci that in smears the latter are found with difficulty, if at all. It is obvious that in the use of the cultural method, organisms of evident pathogenicity, such as spirilli, spirochetes and fusiformed organisms have been disregarded in making the diagnosis; more easily grown organisms having been accepted as the active agents. Cocci obtained by culture under these conditions have been manufactured into vaccines to be used in the treatment of periodontal disorders, a procedure based on mistaken premises.

Realizing our inability to secure cultures from the organisms most commonly found, direct examinations by means of smears were undertaken in twenty untreated, long-standing gingivitis cases. Only one spread of subgingival deposit was made from each patient. It appeared that this procedure, applied in a sufficient number of cases, would give a cross section of the germ types conspicuous in gingivitis The examinations verified in part experiences in an earlier examination of eight gingivitis smears and confirmed the impressions gained in the observation of hundreds of such smears during the past twenty

years. The spirillum proved to be the most conspicuous organism. Of the 8 cases mentioned, 7 showed a preponderance of the *Spirillum sputigenum* and in only one were diplo- and staphylococci most numerous.[3] In the experiment reported here, 12 featured the spirillum, 6 the spirochetes and 2 the fusiform bacillus. Spirochetes were present in all cases, generally in great numbers, and fusiformed bacilli were well represented in 11 slides. The fact that the *Spirillum sputigenum* abounds in such a large number of cases and is found, apparently in almost pure culture, in pulpal and alveolar abscesses, attests to its pathogenicity.

In no case was an actual count made of the organisms, but in each instance, the organism placed at the head of the list appeared to be the most plentiful and was always present in profusion. The experiments show that the specific mouth organisms are not transients, that they dominate the field and that they are responsible for the pathological changes. As none of the varieties belonging to the domiciled contingent in inflamed subgingival spaces respond to ordinary methods of culture, further study is necessary to remove grave doubts concerning their affiliations. Some of them undoubtedly undergo form changes during their developmental cycle,—they are in fact, pleomorphic. W. D. Miller believed that the spirillum is formed by a segmentation of the spirochete; Tunicliffe, that the spirochete is formed from the fusiform bacillus. It is believed (5b) that the fusiform bacilli are products of the sporogeny of long or short thread forms. The solution of the mysteries should be sought in the smears. Studies of the organisms in their native habitat undertaken with the aid of various stains, photomicrographs and camera lucida drawings should precede culture attempts. Notwithstanding reams of descriptions, the morphological peculiarities of the spirilli, the spirochetes and the fusiformed organisms are only incompletely known.

During the past twenty-five years, few students have made more than cursory contributions to this phase of the work. The work of R. D. and Marguerite Dean (6), Aisenberg (7), Coolidge (8) and B. G. Bibby, (unpublished) and M. K. Hine (unpublished) should be noted.

[3] Reported at the 1934 meeting of the Ohio State Dental Society.

SUMMARY

1. The *Spirillum sputigenum* should be ranked as an important factor in oral inflamations.

2. The nomenclature applied during W. D. Miller's time and shortly thereafter should be retained until exhaustive morphological studied justify a change. Changes in terminology, in the present state of our knowledge can be only temporary.

3. The therapeutic use of autogenous vaccines made from cocci grown from lesions in which non-cultivatable mouth organisms abound, appears illogical and unscientific.

REFERENCES TO LITERATURE

1. Hartzell, Thomas B. *J. A. D. A.* 22: 1899, 1935.
2. Miller, W. D. Microorganismen der Mundhoehle, Thieme, Leipzig, 1892, p. 64.
3. Ehrenberg, quoted from C. Fluegge. Mikroorganismen, II Band, Bogel, Leipzig, 1896, p. 67.
4. Hoffmann, E. und von Prowazek. *Cent. Bakt.*, Orig. 41: 741 and 817, 1906.
5. Beust, Theo. B. *J. A. D. A.* 16: 1415, 1929.
5b. Beust, Theo. B. *Den. Cosmos* 50: 594, 1908.
6. Dean, R. W. and Dean, M. *J. Dent. Res.* 11: 759, 1931.
7. Aisenberg, M. S. *Den. Cosmos* 75: 546, 1933.
8. Coolidge, E. D. *Ill. Den. J.* 2: 1933.